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(21) International Application Number: PCT/US93/08743 (22) International Filing Date: 14 September 1993 (14.09.93) (30) Priority data: 07/946,233 <u>14 September 1992 (14.09.92)</u> US (71) Applicant: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US). (72) Inventor: GEORGOPOULOS, Katia ; 28 R.C. Kelley Street, Cambridge, MA 02138 (US). (74) Agents: MYERS, Louis et al.; Lahive & Cockfield, 60 State Street, Boston, MA 02019 (US).		(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: IKAROS: A T CELL PATHWAY REGULATORY GENE (57) Abstract Purified DNA including a sequence encoding an Ikaros protein.		

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IKAROS: A T CELL PATHWAY REGULATORY GENE

Background of the Invention

The invention relates to the Ikaros gene and to the differentiation and generation of T cells.

The generation of the T cell repertoire from a progenitor stem cell proceeds through a differentiation pathway in which the later intrathymic steps are well documented while the early extrathymic events are only poorly characterized. One of the earliest definitive T cell differentiation markers is the CD3 δ gene of the CD3/TCR complex.

Summary of the Invention

The Ikaros gene, a gene active in the early differentiation of T cells, has been discovered. The gene encodes a family of unique zinc finger proteins, the Ikaros proteins. The proteins of the Ikaros family are isoforms which arise from differential splicing of Ikaros gene transcripts. The isoforms of the Ikaros family generally include a common 3' exon (Ikaros exon E7, which includes amino acid residues 203-431 of mouse Ikaros) but differ in the 5' region. The Ikaros family includes all splicing variants which arise from transcription and processing of the Ikaros gene. Five isoforms are described herein. Ikaros proteins can bind and activate the enhancer of the CD3 δ gene and are restricted primarily if not solely to T cells in the adult. The expression pattern of this transcription factor during embryonic development suggests that Ikaros proteins play a role as a genetic switch regulating entry into the T cell lineage. The Ikaros gene is also expressed in the proximal corpus striatum during early embryogenesis in mice.

In general, the invention features, a DNA, preferably a purified DNA, including (or consisting essentially of) a sequence which encodes a peptide including (or consisting essentially of) one or more Ikaros exons. In preferred embodiments the Ikaros exon is any of E1/2, E3, E4, E5, E6, or E7; the purified DNA does not encode exon E7.

In other preferred embodiments: the encoded peptide further includes a second Ikaros exon; the second exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7 and the second exon is any of E1/2, E3, E4, E5, E6.

In other preferred embodiments: the encoded peptide further includes a third Ikaros exon; the third exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7, said second exon is E3, and the third exon is E1/2; the peptide is Ikaros isoform 5.

In other preferred embodiments: the encoded peptide further includes a fourth Ikaros exon; the fourth exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7, the second exon is E6, the third exon is E4, and the fourth exon is E1/2; the first exon is E7, the second exon is E4, the third exon is E3, and the fourth exon is E1/2; the peptide is Ikaros isoform 3 or 4.

In other preferred embodiments: the encoded peptide further includes a fifth Ikaros exon; the fifth exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7, the second exon is E6, the third exon is E5, the fourth exon is E4, and the fifth exon is E1/2; the peptide is Ikaros isoform 2.

In preferred embodiments: the encoded peptide further includes a sixth Ikaros exon; the sixth exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7, the second exon is E6, the third exon is E5, the fourth exon is E4, the fifth exon is E3, and the sixth exon is E1/2; the peptide is Ikaros isoform 1.

In preferred embodiments: the sequence of the encoded Ikaros exon is essentially the same as that of a naturally occurring Ikaros exon, or a fragment thereof having Ikaros activity; the DNA sequence which encodes the Ikaros exon is at least 85%, more preferably at least 90%, yet more preferably at least 95%, and most preferably at least 98 or 99% homologous with DNA encoding a naturally occurring Ikaros exon, or a fragment thereof having Ikaros activity, e.g., Ikaros exon encoding DNA from SEQ ID NO:2 or SEQ ID NO:3; the sequence which encodes an Ikaros exon hybridizes under high or low stringency to a nucleic acid which encodes a naturally occurring Ikaros exon, or a fragment thereof having Ikaros activity, e.g., an Ikaros exon with the same, or essentially the same, amino acid sequence as an Ikaros exon of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5; the amino acid sequence of the encoded Ikaros exon is at least 30, more preferably at least 40, more preferably at least 50, and most preferably at least 60, 80, 100, or 200 amino acid residues in length; the encoded Ikaros amino acid sequence is at least 50% more preferably 60%, more preferably 70%, more preferably 80%, more preferably 90%, and most preferably 95% as long as a naturally occurring Ikaros exon, or a fragment thereof having Ikaros activity; the encoded Ikaros exon is essentially equal in length to a naturally occurring Ikaros exon, or a fragment thereof having Ikaros activity; the amino acid sequence of the encoded Ikaros exon is at least 80%, more preferably at least 85%, yet more preferably at least 90%, yet more preferably at least 95%, and a most preferably at least 98 or 99% homologous with a naturally occurring Ikaros exon sequence, or a fragment thereof having Ikaros activity, e.g., an Ikaros exon sequence of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5; the encoded Ikaros exon amino acid sequence

is the same, or essentially the same, as that of a naturally occurring Ikaros exon, or a fragment of the sequence thereof, e.g., an Ikaros exon described in SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5; and the peptide has Ikaros peptide activity.

In preferred embodiments: the exons in the encoded peptide are arranged in the same relative linear order as found in a naturally occurring isoform, e.g., Ikaros isoform 1, e.g., in a peptide having the exons E3 and E7, E3 is located N-terminal to E7; the linear order of the encoded exons is different from that found in a naturally occurring isoform, e.g., in Ikaros isoform 1, e.g., in a peptide having exons E3, E5, and E7, the direction N-terminal to C-terminal end, is E5, E3, E7; the exons in the encoded peptide differ in one or more of composition (i.e., which exons are present), linear order, or number (i.e., how many exons are present or how many times a given exon is present) from a naturally occurring Ikaros isoform, e.g., from Ikaros isoform 1, 2, 3, 4, or 5.

In another aspect, the invention features, a peptide, preferably a substantially pure peptide, including (or consisting essentially of) one or more Ikaros exons. In preferred embodiments the Ikaros exon is E1/2, E3, E4, E5, E6, or E7; the peptide does not include exon E7.

In other preferred embodiments: the peptide further includes a second Ikaros exon; the second exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7 and the second exon is any of E1/2, E3, E4, E5, E6.

In other preferred embodiments: the peptide further includes a third Ikaros exon; the third exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7, the second exon is E3, and the third exon is E1/2; the peptide is Ikaros isoform 5.

In other preferred embodiments: the peptide further includes a fourth Ikaros exon; the fourth exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7, the second exon is E4, the third exon is E3, and the fourth exon is E1/2; the first exon is E7, the second exon is E4, the third exon is E3, and the fourth exon is E1/2; the peptide is Ikaros isoform 3 or 4.

In other preferred embodiments the peptide further includes a fifth Ikaros exon; the fifth exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7, the second exon is E6, the third exon is E5, the fourth exon is E4, and the fifth exon is E1/2; the peptide is Ikaros Isoform 2.

In other preferred embodiments the peptide further includes a sixth Ikaros exon; the sixth exon is any of E1/2, E3, E4, E5, E6, or E7; the first exon is E7, the second exon is E6, the third exon is E5, the fourth exon is E4, the fifth exon is E3, and the sixth exon is E1/2; the peptide is Ikaros isoform 1.

In preferred embodiments: the sequence of the Ikaros exon is essentially the same as that of a naturally occurring Ikaros exon, or a fragment thereof having Ikaros activity; the amino acid sequence of the Ikaros exon is such that a nucleic acid

sequence which encodes it is at least 85%, more preferably at least 90%, yet more preferably at least 95%, and most preferably at least 98 or 99% homologous with DNA encoding a naturally occurring Ikaros exon, or a fragment thereof having Ikaros activity, e.g., Ikaros exon encoding DNA from SEQ ID NO:2 or SEQ ID NO:3; the amino acid sequence of the Ikaros exon is such that a nucleic acid sequence which encodes it hybridizes under high or low stringency to a nucleic acid which encodes a naturally occurring Ikaros exon, or a fragment thereof having Ikaros activity, e.g., an Ikaros exon with the same, or essentially the same, amino acid sequence as an Ikaros exon of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5; the amino acid sequence of the Ikaros exon is at least 30, more preferably at least 40, more preferably at least 50, and most preferably at least 60, 80, 100, or 200 amino acid residues in length; the encoded Ikaros amino acid sequence is at least 50% more preferably 60%, more preferably 70%, more preferably 80%, more preferably 90%, and most preferably 95% as long as a naturally occurring Ikaros exon, or a fragment thereof having Ikaros activity; the Ikaros exon is essentially equal in length to a naturally occurring Ikaros exon; the amino acid sequence of the Ikaros exon is at least 80%, more preferably at least 85%, yet more preferably at least 90%, yet more preferably at least 95%, and a most preferably at least 98 or 99% homologous with a naturally occurring Ikaros exon sequence, or a fragment thereof having Ikaros activity, e.g., an Ikaros exon sequence of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5; the Ikaros exon amino acid sequence is the same, or essentially the same, as that of a naturally occurring Ikaros exon, or a fragment of the sequence thereof, e.g., an Ikaros exon described in SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5; and the peptide has Ikaros peptide activity.

In preferred embodiments: the exons in the peptide are arranged in the same relative linear order as found in a naturally occurring isoform, e.g., in Ikaros isoform 1, e.g., in a peptide having the exons E3 and E7, E3 is located N-terminal to E7; the linear order of the exons is different from that found in a naturally occurring isoform, e.g., in Ikaros isoform 1, e.g., in a peptide having exons E3, E5, and E7, the direction N-terminal to C-terminal end, is E5, E3, E7; the exons in the peptide differ in one or more of composition (i.e., which exons are present), linear order, or number (i.e., how many exons are present or how many times a given exon is present) from a naturally occurring Ikaros isoform, e.g., from Ikaros isoform 1, 2, 3, 4, or 5.

In another aspect, the invention features, a DNA, preferably a purified DNA, which includes (or consists essentially of) a DNA sequence encoding an Ikaros peptide, e.g., an Ikaros peptide having Ikaros activity, e.g., Ikaros isoform 1, 2, 3, 4, or 5. In preferred embodiments: the sequence of the encoded Ikaros peptide is essentially the same as the sequence of a naturally occurring Ikaros peptide, or a fragment thereof having Ikaros activity; the DNA sequence is at least 85%, more preferably at least 90%, yet more preferably at least 95%, and most preferably at least

98 or 99% homologous with DNA encoding a naturally occurring Ikaros peptide, or a fragment thereof having Ikaros activity; e.g., with DNA from SEQ ID NO:2 or SEQ ID NO:3; the amino acid sequence of the encoded peptide is such that it can be encoded by a nucleic acid which hybridizes under high or low stringency conditions to a nucleic acid which encodes a peptide with the same, or essentially the same, amino acid sequence as the peptide of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5; the encoded peptide is at least 30, more preferably at least 40, more preferably at least 50, and most preferably at least 60, 80, 100, or 200 amino acid residues in length; the encoded peptide is at least 50% more preferably at least 60%, more preferably 70%, more preferably 80%, more preferably 90%, and most preferably 95% as long as a naturally occurring Ikaros peptide, or a fragment thereof having Ikaros activity; the encoded peptide is essentially the same length as a naturally occurring Ikaros peptide, or a fragment thereof having Ikaros activity; the encoded peptide is at least 80%, more preferably at least 85%, yet more preferably at least 90%, yet more preferably at least 95%, and a most preferably at least 98 or 99% homologous with an amino acid sequence which is the same, or essentially the same, as a naturally occurring Ikaros peptide, or a fragment thereof having Ikaros activity, e.g., the peptide sequence of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5; and, the amino acid sequence of the peptide is essentially the same as the sequence of a naturally occurring Ikaros peptide, or a fragment thereof having Ikaros activity, e.g., the sequence, described in SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5.

In another aspect, the invention features, a DNA, preferably a purified DNA, which includes (or consists essentially of) a sequence encoding a peptide of 20 or more amino acids in length, the peptide having at least 90% homology with an amino acid sequence which is the same, or essentially the same, as a naturally occurring Ikaros peptide, e.g., the amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5. In preferred embodiments the purified DNA encodes: a peptide which is at least 30, more preferably at least 40, more preferably at least 50, and most preferably at least 60, 80, 100, or 200 , amino acid residues in length; the encoded peptide is at least 50% more preferably at least 60%, more preferably 70%, more preferably 80%, more preferably 90%, and most preferably 95% as long as a naturally occurring Ikaros peptide, or fragment thereof having Ikaros activity; the encoded peptide is essentially the same length as a naturally occurring Ikaros peptide; a peptide which is at least 80, more preferably at least 85, yet more preferably at least 90, yet more preferably at least 95, and most preferably at least 98 or 99% homologous with an amino acid sequence which is the same, or essentially the same, as a naturally occurring Ikaros peptide, or a fragment thereof having Ikaros activity, e.g., as the amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5; and, a peptide with Ikaros activity.

In another aspect, the invention features, a DNA, preferably a purified DNA, which includes (or consists essentially of) a DNA sequence which hybridizes under high or low stringency to a nucleic acid which encodes a peptide with the same, or essentially the same, amino acid sequence as a naturally occurring Ikaros peptide, e.g., the peptide of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5. In preferred embodiments: the DNA sequence is at least 85%, more preferably at least 90%, yet more preferably at least 95%, and most preferably at least 98 or 99% homologous with DNA encoding a naturally occurring Ikaros peptide, or a fragment thereof having Ikaros activity, e.g., with DNA from of SEQ ID NO:2 or SEQ ID NO:3; the purified DNA encodes a peptide at least 30, more preferably at least 40, more preferably at least 50, and most preferably at least 60, 80, 100, or 200 amino acid residues in length; the encoded peptide is at least 50% more preferably at least 60%, more preferably 70%, more preferably 80%, more preferably 90%, and most preferably 95% as long as a naturally occurring Ikaros peptide, or fragment thereof having Ikaros activity; the encoded peptide is essentially the same length as a naturally occurring Ikaros peptide; the purified DNA encodes a peptide at least 80, more preferably at least 85, yet more preferably at least 90, yet more preferably at least 95, and most preferably at least 98 or 99% homologous with an amino acid sequence which is the same, or essentially the same, as a naturally occurring Ikaros peptide, e.g., the amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5; and, the purified DNA encodes a peptide having essentially the same amino acid sequence, or a fragment of the amino acid sequence, described in SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5.

In another aspect, the invention includes a vector which includes DNA of the invention, preferably a purified DNA of the invention, which encodes a peptide of the invention.

The invention also includes: a cell, e.g., a cultured cell or a stem cell, containing purified Ikaros-protein-encoding-DNA; a cell capable of expressing an Ikaros protein; a cell capable of giving rise to a transgenic animal or to a homogeneous population of hemopoietic cells, e.g., lymphoid cells, e.g., T cells; an essentially homogeneous population of cells, each of which includes purified Ikaros-protein-encoding-DNA; and a method for manufacture of a peptide of the invention including culturing a cell which includes a DNA, preferably a purified DNA, of the invention in a medium to express the peptide.

In another aspect, the invention features a peptide of the invention, preferably a substantially pure peptide of the invention, e.g.: a peptide having Ikaros activity, e.g., Ikaros isoform 1, 2, 3, 4, or 5. In preferred embodiments: the sequence of the encoded Ikaros peptide is essentially the same as the sequence of a naturally occurring Ikaros peptide, or a fragment thereof having Ikaros activity; the sequence of the peptide is such that it is encoded by a DNA sequence at least 85%, more preferably at

least 90%, yet more preferably at least 95%, and most preferably at least 98 or 99% homologous with DNA encoding a naturally occurring Ikaros peptide, or a fragment thereof having Ikaros activity; e.g., with DNA from SEQ ID NO:2 or SEQ ID NO:3; the amino acid sequence of the peptide having Ikaros activity is such that it can be encoded by a nucleic acid which hybridizes under high or low stringency conditions to a nucleic acid which encodes a peptide with the same, or essentially the same, amino acid sequence as the peptide of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5; the peptide is at least 30, more preferably at least 40, more preferably at least 50, and most preferably at least 60, 80, 100, or 200 amino acid residues in length; the peptide is at least 50% more preferably at least 60%, more preferably 70%, more preferably 80%, more preferably 90%, and most preferably 95% as long as a naturally occurring Ikaros peptide, or fragment thereof having Ikaros activity; the peptide is essentially the same length as a naturally occurring Ikaros peptide, or a fragment thereof having Ikaros activity; the peptide is at least 80%, more preferably at least 85%, yet more preferably at least 90%, yet more preferably at least 95%, and most preferably at least 98 or 99% homologous with an amino acid sequence which is the same, or essentially the same, as a naturally occurring Ikaros peptide, or a fragment thereof having Ikaros activity, e.g., the peptide sequence of SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5; and, the amino acid sequence of the peptide is essentially the same as the sequence of a naturally occurring Ikaros peptide, or a fragment thereof having Ikaros activity, e.g., the sequence, described in SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5.

In preferred embodiments a peptide of the invention, preferably a purified peptide of the invention, is produced by expression of a DNA of the invention, preferably a purified DNA of the invention.

The invention also includes substantially pure preparation of an antibody, preferably a monoclonal antibody directed against an Ikaros protein; a therapeutic composition including an Ikaros protein and a pharmaceutically acceptable carrier; a therapeutic composition which includes a purified DNA of the invention and a pharmaceutically acceptable carrier.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse, including administering a therapeutically-effective amount of an Ikaros peptide to the animal.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse including administering to the animal cells selected, e.g., selected in vitro, for the expression of a product of the Ikaros gene, e.g., hematopoietic stem cells, e.g., cells

transformed with Ikaros-peptide-encoding DNA, e.g., hematopoietic stem cells transformed with Ikaros-peptide-encoding DNA.

In preferred embodiments: the cells are taken from the animal to which they are administered; the cells are taken from an animal which is MHC matched with the animal to which they are administered; the cells are taken from an animal which is syngeneic with the animal to which they are administered; the cells are taken from an animal which is of the same species as is the animal to which they are administered.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse, including administering to the animal a nucleic acid encoding an Ikaros peptide and expressing the nucleic acid.

In another aspect, the invention features a method of evaluating the effect of a treatment, e.g., a treatment designed to promote or inhibit hematopoiesis, including carrying out the treatment and evaluating the effect of the treatment on the expression of the Ikaros gene.

In preferred embodiments the treatment is administered: to an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for an immune system disorder, e.g., a T or B cell related disorder, e.g., a nude mouse or a SCID mouse, or a cell, e.g., a cultured stem cell.

In another aspect, the invention features a method for determining if a subject, e.g., a human, is at risk for a disorder related to mis-expression of the Ikaros gene, e.g., a leukemic disorder or other disorder of the immune system, e.g., an immunodeficiency, or a T or B cell related disorder, e.g., a disorder characterized by a shortage of T or B cells, including examining the subject for the expression of the Ikaros gene, non-wild type expression or mis-expression being indicative of risk.

In another aspect, the invention features a method for determining if a subject, e.g., a human, is at risk for a disorder related to mis-expression of the Ikaros gene, e.g., a leukemic disorder or other disorder of the immune system, e.g., an immunodeficiency, or a T or B cell related disorder, e.g., a disorder characterized by a shortage of T or B cells, including providing a nucleic acid sample from the subject and determining if the structure of an Ikaros gene allele of the subject differs from wild type.

In preferred embodiments: the determination includes determining if an Ikaros gene allele of the subject has a gross chromosomal rearrangement; the determination includes sequencing the subject's Ikaros gene.

In another aspect, the invention features, a method of evaluating an animal or cell model for an immune disorder, e.g., a T cell related disorder, e.g., a disorder characterized by a shortage of T or B cells, including determining if the Ikaros gene in

the animal or cell model is expressed at a predetermined level or if the Ikaros gene is mis-expressed. In preferred embodiments: the predetermined level is lower than the level in a wild type or normal animal; the predetermined level is higher than the level in a wild type or normal animal; or the pattern of isoform expression is altered from wildtype.

In another aspect, the invention features a transgenic rodent, e.g., a mouse, having a transgene which includes an Ikaros gene or Ikaros protein encoding DNA. In preferred embodiments: the Ikaros gene or DNA includes a deletion, e.g. a deletion of all or part of one or more Ikaros exons, e.g., a deletion of all or part of exon E7 or a deletion of all or part of exons E3 or E4, or is otherwise mis-expressed.

In another aspect, the invention features a method of expressing a heterologous gene, e.g., in a cell e.g., a stem cell, including placing the gene under the control of an Ikaros-responsive control element, and contacting the Ikaros-responsive control element with an Ikaros protein.

In preferred embodiments: the Ikaros-responsive control element includes an enhancer, e.g., an δA element, an NFkB element, or one of the Ikaros binding sequences, e.g., one of the consensus sequences, disclosed herein; the Ikaros-responsive control element includes the regulatory region of the CD3 δ gene; the heterologous gene and the Ikaros-responsive control element are carried on a vector; the method further includes the step of transforming a cell with a vector which includes a heterologous gene under the control of an Ikaros-responsive control agent; the heterologous gene is expressed in a cell which normally includes or expresses an Ikaros protein.

In another aspect, the invention features a method of expressing a gene under the control of an Ikaros-responsive control element in a cell including administering an Ikaros protein to the cell.

In preferred embodiments: the method further includes transforming the cell with DNA which encodes an Ikaros protein to supply an Ikaros protein; the gene is a heterologous gene.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for a disorder of the nervous system, e.g., a disorder of the corpus striatum, e.g., Alzheimer's disease, immune system disorder, including administering a therapeutically effective amount of an Ikaros protein to the animal.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for a disorder of the nervous system, e.g., a disorder of the corpus striatum, e.g., Alzheimer's disease, including administering to the animal cells selected, e.g., selected in vitro, for the expression of a product of the Ikaros gene, e.g., hematopoietic stem cells, e.g., cells transformed

with Ikaros-protein-encoding DNA, e.g., hematopoietic stem cells transformed with Ikaros-protein-encoding DNA.

In preferred embodiments: the cells are taken from the animal to which they are administered; the cells are taken from an animal which is MHC matched with the animal to which they are administered; the cells are taken from an animal which is syngeneic with the animal to which they are administered; the cells are taken from an animal which is of the same species as is the animal to which they are administered.

In another aspect, the invention features a method for treating an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for a disorder of the nervous system, e.g., a disorder of the corpus striatum, e.g., Alzheimer's disease, including administering to the animal a nucleic acid encoding an Ikaros peptide and expressing the nucleic acid.

In another aspect, the invention features a method of evaluating the effect of a treatment for a disorder of the nervous system, e.g., a disorder of the corpus striatum, e.g., Alzheimer's disease, including administering the treatment and evaluating the effect of the treatment on the expression of the Ikaros gene.

In preferred embodiments the treatment is administered: to an animal, e.g., a human, a mouse, a transgenic animal, or an animal model for a disorder of the nervous system, e.g., a disorder of the corpus striatum, e.g., Alzheimer's disease, or a cell, e.g., a cultured stem cell.

In another aspect, the invention features a method for determining if a subject, e.g., a human, is at risk for a disorder related to mis-expression of the Ikaros gene, e.g., a disorder of the nervous system, e.g., a disorder of the corpus striatum, e.g., Alzheimer's disease, including examining the subject for the expression of the Ikaros gene, non-wild type expression or mis-expression being indicative of risk.

In another aspect, the invention features a method for determining if a subject, e.g., a human, is at risk for a disorder related to mis-expression of the Ikaros gene, e.g., a disorder of the nervous system, e.g., a disorder of the corpus striatum, e.g., Alzheimer's disease, including providing a nucleic acid sample from the subject and determining if the structure of an Ikaros gene allele of the subject differs from wild type.

In preferred embodiments: the determination includes determining if an Ikaros gene allele of the subject has a gross chromosomal rearrangement; the determination includes sequencing the subject's Ikaros gene.

In another aspect, the invention features, a method of evaluating an animal or cell model for a disorder of the nervous system, e.g., a disorder of the corpus striatum, e.g., Alzheimer's disease, including determining if the Ikaros gene in the animal or cell model is expressed at a predetermined level or if the Ikaros gene is mis-expressed.

In preferred embodiments: the predetermined level is lower than the level in a wild type or normal animal; the predetermined level is higher than the level in a wild type or normal animal.

In another aspect, the invention features, a method of inhibiting an interaction, e.g., binding, between a protein, e.g., a first Ikaros isoform, and a DNA sequence, e.g., a DNA sequence under the control of a δA sequence, an NKFB sequence, a sequence which corresponds to an Ikaros binding oligonucleotide described herein, or a site present in the control region of a lymphocyte restricted gene, e.g., TCR- α , - β , or - δ , CD3 - δ , - ϵ , - γ genes, the SL3 gene, or the HIV LTR gene. The methods includes contacting the DNA sequence with an effective amount of a second Ikaros isoform, or with a DNA binding fragment of an Ikaros isoform, e.g., of the second Ikaros isoform.

In preferred embodiments the fragment is deleted for all or part of an Ikaros exon, e.g., for all or part of E1/2, E3, E4, E5, E6, or E7.

In another aspect, the invention features, a method of inhibiting an interaction, e.g., binding, between a protein, e.g., a first Ikaros isoform, and a DNA sequence, e.g., a δA sequence, an NKFB sequence, a sequence which corresponds to an Ikaros binding oligonucleotide described herein, or a site present in the control region of a lymphocyte restricted gene, e.g., TCR- α , - β , or - δ , CD3 - δ , - ϵ , - γ genes, the SL3 gene, or the HIV LTR gene. The methods includes contacting the protein with an effective amount of an Ikaros binding oligonucleotide. In preferred embodiments the oligonucleotide includes a sequence chosen from, IK-BS1, IK-BS2, IK-BS3, IK-BS4, or IK-BS5.

In preferred embodiments: the oligonucleotide preferentially binds to a first Ikaros isoform; the oligonucleotide preferentially binds to a second Ikaros isoform.

In another aspect the invention includes an Ikaros binding oligonucleotide, e.g., IK-BS1, IK-BS2, IK-BS3, IK-BS4, or IK-BS5. In preferred embodiments the oligonucleotide contains at least two, three, four, or five copies of one of the Ikaros binding oligonucleotide sequences disclosed herein.

In another aspect, the invention features a method of attenuating the binding of a first Ikaros isoform to target DNA. The method includes contacting the target DNA with an effective amount of a second Ikaros isoform, or with a DNA binding fragment of said second isoform.

Heterologous gene, as used herein, is a gene which is not normally under the control of an Ikaros responsive control element.

An Ikaros-responsive control element, as used herein is a region of DNA which, when present upstream or downstream from a gene, results in regulation, e.g., increased transcription of the gene in the presence of an Ikaros protein.

Purified DNA is DNA that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (i.e., one at the 5' end and

one at the 3' end) in the naturally occurring genome of the organism from which the DNA of the invention is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other DNA sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

Homologous refers to the sequence similarity between two polypeptide molecules or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomeric subunit, e.g., if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences. For example, 6 of 10, of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGGC share 50% homology.

A transgene is defined as a piece of DNA which is inserted by artifice into a cell and becomes a part of the genome of the animal which develops in whole or part from that cell. Such a transgene may be partly or entirely heterologous to the transgenic animal.

A transgenic animal, e.g., a transgenic mouse, is an animal having cells that contain a transgene, which transgene was introduced into the animal, or an ancestor of the animal, at a prenatal, e.g., an embryonic stage.

An enhancer region is defined as a cis-acting DNA sequence capable of increasing transcription from a promoter that is located either upstream or downstream of the enhancer region. Such DNA sequences are well known to those skilled in the art of eukaryotic gene expression.

A substantially pure preparation of a peptide is a preparation which is substantially free of the peptides with which it naturally occurs in a cell. A substantially pure preparation of a non-naturally occurring peptide is one which is at least 10% by weight of the peptide of interest.

Mis-expression, as used herein, refers to a non-wild type pattern of gene expression. It includes: expression at non-wild type levels, i.e., over or under expression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of the tissue specificity of expression, e.g., increased or decreased expression (as compared with wild type) in a

predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the size, amino acid sequence, post-translational modification, or a biological activity of an Ikaros gene product; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus; or a pattern of isoform expression which differs from wildtype.

The terms peptide, protein, and polypeptide are used interchangeably herein.

A peptide has Ikaros activity if it has one or more of the following properties: the ability to stimulate transcription of a DNA sequence under the control any of a δA element, an NF κ B element, or one of the Ikaros binding oligonucleotide consensus sequences disclosed herein; the ability to bind to any of a δA element, an NF κ B element, or one of the Ikaros binding oligonucleotide consensus sequences disclosed herein; or the ability to competitively inhibit the binding of a naturally occurring Ikaros isoform to any of a δA element, an NF κ B element, or one of the Ikaros binding oligonucleotide consensus sequences disclosed herein. An Ikaros peptide is a peptide with Ikaros activity.

The invention is useful for identifying T cells; identifying cells which can develop into T cells; and generally, in the investigation of hemopoiesis, e.g., in the differentiation of progenitor stem cells into T cells. The role of the Ikaros gene and its products can be studied, e.g., in cells, e.g., cultured cells, transformed with the Ikaros gene or fragments thereof, or in transgenic animals. The invention is also useful for: promoting the expression of markers of cell lineage, e.g., CD3 δ genes; enhancing the ability of a cell, e.g., a stem cell, to develop into a T cell; screening individuals at risk for genetic T cell disorders, e.g., leukemia; and treating immune disorders (e.g., immunodeficiencies, e.g., AIDS, or chemical, drug, or radiation induced immunodeficiencies, or cancers, e.g., leukemia) characterized by a shortage of T cells; for investigating the structure and expression of the Ikaros gene or iso forms of the gene product; for investigating species or tissue differences in the expression of the Ikaros gene or its isoforms; for investigating the structure and function of DNA binding proteins; for studying the structure and function of zinc finger containing proteins; for the construction of transgenic animals; for inhibiting the binding of Ikaros to a target molecule; for studying the relative affinities of Ikaros isoforms for target DNA; and for searching for or manipulating the expression of genes under the control of Ikaros isoforms.

Other features and advantages of the invention will be apparent from the following description and from the claims.

Detailed Description

The drawings are first briefly described.

Drawings

Fig. 1A is a map of the δ A element of the CD3 enhancer (SEQ ID NO:1).

Fig. 1B is a graph of the contribution of the CRE and the G box to the activity of the element as analyzed by expression of tkCAT reporter gene under the control of various element sequences.

Fig. 1C is a graph of the effect of Ikaros expression on the activity of the δ element in non-T cells.

Fig. 2 is a map of the DNA sequence of a murine Ikaros cDNA and the desired amino acid sequence encoded thereby (SEQ ID NO:2).

Fig. 3 is a partial sequence of a human Ikaros cDNA (SEQ ID NO:3).

Fig. 4 is a depiction of the amino acid composition of the IK-1 cDNA (SEQ ID NO:5). (IK-1 is comprised of all six coding exons (Ex1/2; Ex3; Ex4; Ex5; Ex6; and Ex7.) An arrow indicates the first amino acid in every exon.

Fig. 5 is a diagram of exon usage in the Ikaros 1-5 cDNAs. Exon numbers are indicated at the bottom left hand corner of each box (Ex). Zinc finger modules are shown on top of the encoding exons (Fx).

Fig. 6 is a depiction of the exon organization at the Ikaros locus indicating primer sets 1/2 and 3/4 used for amplification of the respective isoforms.

Fig. 7 is a map of the genomic organization of the mouse Ikaros gene. The entire gene is 80-90 kB in length. Intronic or uncharacterized DNA is indicated as a line between 5' and 3'. Exons are indicated as boxes. Lines numbered f2, f10, f4, and f8 indicate phage inserts corresponding to the sequence immediately above. Restriction sites are indicated by the usual abbreviations.

Fig. 8 is a model of Ikaros isoform control of differential gene expressions. Th=thymus; Sp=spleen; Ex=day of embryonic development; Dx = day of postnatal life. The left hand column represents the relative expression of an isoform at a given developmental stage. Open bar=Ik-1; Horizontal stripes=Ik-2; Diagonal stripes=Ik-3; and solid bar=Ik-4. The right hand side shows the resulting reactivity of Ikaros binding sites at a given developmental stage. Light bars=low affinity sites (sites at which isoforms 1, 2, 3 and 4 bind with similar affinities); Dark bars=high affinity inverted or direct repeat containing sites (e.g., NFkB sites, Ik1-4 bind with high affinity); Diagonal bars=single high affinity sites (sites where Ik1 and Ik2 bind but Ik3 and Ik4 don't bind (and therefore won't attenuate the binding of Ik-1 and Ik-2).

Ikaros: A master regulator of hemopoietic differentiation

A hemopoietic stem cell in the appropriate microenvironment will commit and differentiate into one of many cell lineages. Signal transduction molecules and transcription factors operating at distinct check points in this developmental pathway

will specify the cell fate of these early progenitors. Such molecules are viewed as master regulators in development but also serve as markers for the ill defined stages of early hemopoiesis.

Studies on the transcriptional mechanisms that underlie gene expression in T and B cells have identified several transcriptional factors involved in lymphocyte differentiation. However, some of these genes appear to play a role in several developmental systems as determined by their non restricted pattern of expression in the adult and in the developing embryo. The HMG box DNA binding proteins TCF and LEF restricted to T cells and early lymphocytes in the adult are widely expressed in the developing embryo. The T cell specific GATA-3 transcription factor is also expressed outside the hemopoietic system in the early embryo. The ets family members Ets-1 and Elf-1 are widely distributed as well. In addition, the binding affinity and transcription potential of most of these proteins is controlled by other tissue restricted molecules. The ets proteins interact with additional factors for high affinity binding to their cognate sequences. TCFI, LEF and ets-1 must interact with other lymphoid restricted accessory proteins to activate transcription.

In search of a lymphoid restricted transcriptional enhancer, in control of gene expression in early T cells, we have isolated the Ikaros gene, which encodes a zinc finger DNA binding protein. In the early embryo, the Ikaros gene is expressed in the hemopoietic liver but from mid to late gestation becomes restricted to the thymus. The only other embryonic site with Ikaros mRNA is a small area in the corpus striatum. In the adult, the Ikaros mRNA is detected only in the thymus and in the spleen (Georgopoulos *et al.* 1992). The Ikaros gene functions as a transcriptional enhancer when ectopically expressed in non lymphoid cells.

The Ikaros gene plays an important role in early lymphocyte and T cell differentiation. The Ikaros gene is abundantly expressed at early embryonic hemopoietic sites is later on restricted in the developing thymus. The thymus together with the spleen are the prime sites of expression in the adult. This highly enriched expression of the Ikaros gene was also found in early and mature primary T cells and cell lines. This restricted pattern of expression of the Ikaros gene at sites where embryonic and adult T cell progenitors originate together with the ability of the encoded protein to activate transcription from the regulatory domain of an early T cell differentiation antigen supported a determining role in T cell specification.

Differential splicing at the Ikaros genomic locus generates at least five transcripts that encode proteins with distinct DNA binding domains. These Ikaros protein isoforms (IK-1, IK-2, IK-3, IK-4, IK-5) have overlapping but also distinct DNA binding specificity dictated by the differential usage of zinc finger modules at their N-terminus. The core binding site for four of the Ikaros proteins is the GGGA motif but outside this sequence their specificity differs dramatically. The IK-3 protein

shows strong preferences for bases at both the 5' and 3' flanking sequences which restricts the number of sites it can bind to. The Ik-1 protein also exhibits strong preference for some of these flanking bases and can bind to wider range of sequences. The Ik-2 protein, the most promiscuous of the three proteins, can bind to sites with just the GGGAa/t motif. Finally, the Ik-4 protein with similar sequences specificity to Ik-1 binds with high affinity only when a second site is in close proximity suggesting cooperative site occupancy by this protein. A number of putative binding sites for the Ikaros proteins were identified in the enhancers of cell T cell receptor δ - β and α and the CD3- δ , ϵ and γ genes, in the HIV LTR in the IL2-R promoter and in a variety of other lymphocyte restricted genes. One of these sites, the NFkB variant in the IL-2R α enhancer, binds all of these proteins with high affinity. This NFkB motif, a crucial regulatory element for expression of the IL2 Receptor α in T cells is a strongly activated by the Ik-1 and Ik-2 isoforms in T and non T cells. The IL-2R α gene is expressed at high levels in activated T cells but also in fetal thymocytes which also express high levels of the Ikaros gene. Thus, gene regulation of at least the IL2 α Receptor during T cell differentiation and activation may be controlled by the intricate interplay of NFkB and Ikaros transcription factors interacting on common grounds.

The embryonic expression pattern and activation potential of the Ikaros isoform is markedly different. The strong transcription factors Ik-1 and Ik-2 are abundantly expressed in the early fetal liver, in the maturing thymus and in a small area of the developing brain while the weak activators Ik-3, Ik-4 are expressed at lower levels. However, since the Ik-1 and particularly the Ik-2 proteins bind a wider range of sites than Ik-3 and Ik-4, the available molecules that bind to the same sequences as Ik-3 and Ik-4 may be at similar concentration. Moreover, in the day 14 embryonic thymus the weak activator Ik-4 is present at equal or greater levels than Ik-1 and Ik-2 isoforms. Competition between Ik-4, Ik-1 and Ik-3 for perfect or imperfect inverted and direct repeats of their recognition motif (NFkB variants) may mediate low levels of transcriptional activation from these sites in the early thymus. Steady state levels of the activating Ikaros factors in combination to the decreasing levels of the non activating Ik-4 protein maturing thymocytes may turn on the activity of these double sites in the developing thymus. This may result in denovo expression of stage specific T cell differentiation antigens. Finally, activation from low affinity binding sites may only be possible in these late stages of T cell differentiation when the Ikaros activating proteins are in excess. Concentration gradients of the *Drosophila* NKkB homologue Dorsal and distinct protein-protein interactions with other nuclear factors is responsible for the activation level and threshold response from low and high affinity binding sites in the fly embryo. Fig. 8 provides a model in which the relative concentrations of Ikaros isoforms at different developmental stages confer different reactivities on the various sites.

The transcriptional activity of the Ik-3 and Ik-4 proteins may be further regulated by T cell restricted signals mediating postranslational modifications or by protein-protein interactions. The Ik-4 protein binds NFkB motif in a cooperative fashion and may therefore interact in situ with other members of the Ikaros or of the NFkB family. These protein-protein-DNA complexes may dictate a differential transcriptional outcome.

The differential expression of the Ikaros isoforms during T cell ontogeny, their overlapping but distinct binding specificity and their diverse transcription potential may be responsible for the orderly activation of stage specific T cell differentiation markers. Multiple layers of gene expression in the developing lymphocyte can be amenable to regulation by these proteins. Synergistic interactions and/or competition between the members of the Ikaros family and other factors in these cells on qualitatively similar and distinct target sites could dictate the genetic make up of the resting and activated lymphocyte. This functional dissection of the Ikaros gene strongly support its proposed function as a master gene in lymphocytes. The role of the Ikaros gene as the necessary genetic switch for early hemopoiesis and T cell development in ultimately being addressed in gene ablation studies in transgenic animals.

Mutational Analysis of the δ Element of the CD3 δ Enhancer

One approach useful for characterizing early events in T cell differentiation is to study the regulation of transcription of T cell restricted antigens. We have chosen the transcriptional control of one of the earliest and definitive T cell differentiation markers, the CD3 δ gene of the CD3/TCR complex. In order to identify a transcription factor expressed at or earlier than T cell commitment which can function as a genetic switch regulating entry into the T cell lineage we have characterized a T cell specific enhancer mediating expression of this gene. This enhancer is comprised of two functionally distinct elements δ A and δ B with activity restricted in T cells. Mutational analysis of the δ A element has further identified two transcriptionally active binding sites, a CRE (Cyclic AMP response)- like element and a G rich sequence motif both of which are required for full activity of the δ A element and the CD3 enhancer, see Fig. 1.

Fig. 1 depicts the functional dissection of the δ A element of the CD3 δ enhancer. Fig. 1A shows the binding sites in the δ element (SEQ ID NO:1). The boxed sequences represent the CRE-like and the G rich motif both important for activity of the δ A element. Mutations introduced in the δ A element are shown below the sequence.

Fig. 1B shows the contribution of the CRE and the G box to the activity of the δ A element and the CD3 δ enhancer as analyzed by transient expression assays in the T cell line EL4. The activity of the tkCAT reporter gene under the control of wild type δ A, δ Amul and δ Amu2 as reiterated elements or in the context of the CD3 δ enhancer

was determined as described in Georgopoulos et al. (1992) Mol. Cell. Biol. 12:747. Reporter gene activation (R.A.) was expressed as the ratio of Chloramphenicol Acetyl Transferase (CAT) to Growth Hormone (GH) activity estimated for each transfection assay. Fig. 1C shows that the expression of the Ikaros gene (Ik-2) in non T cells upregulates the activity of the δ A element. The CDM8 and CDM8:Ikaros recombinant I expression vectors were cotransfected with the tkcat 3 δ A, tkcat 3 δ Amul, tkcat 3 δ Amu2 and tkcat δ enhancer reporter genes in CV1 (kidney epithelial) cells as described in Georgopoulos et al. (1992). The ratio of reporter activation (R.A.=CAT/GH) in the presence and absence of Ikaros expression was estimated. Three isoforms of the ubiquitously expressed CRE-Binding Protein were cloned from T cells for their ability to interact with the CRE-like binding site of the δ A element, see Georgopoulos et al. (1992). Although dominant negative mutants of this protein down regulate the activity of this enhancer element in T cells, expression of this transcription factor in all hemopoietic and non hemopoietic cells argues against it being the switch that activates the CD3 δ enhancer in the early prothymocyte progenitor. A variant of the δ A element (δ Amul-CRE) was used to screen a T cell expression library as described in Georgopoulos et al. (1992). As described below, a T cell restricted cDNA was cloned encoding for a novel zinc finger protein (Ikaros) that binds to the G box of the A element.

Cloning the Ikaros Gene

A T cell expression cDNA library from the mature T cell line E14 was constructed into the A ZAP phage vector.

A multimerized oligonucleotide encoding sequence (SEQ ID NO:4) from one of the protein binding sites of the CD38 enhancer was used as a radiolabelled probe to screen this expression library for the T cell specific proteins that bind and mediate enhancer function by the southwestern protocol of Singh and McKnight. Four gene encoding DNA binding proteins were isolated. One, the Ikaros gene, encoded a T cell specific protein.

The Sequence of Ikaros

The sequence of the Ikaros gene was determined using the Sanger dideoxyl sequencing protocol. The derived amino acid sequence was determined using the MAP program of GCG (available from the University of Wisconsin) and Strider sequence analysis programs. Fig. 2 provides the sequence of an Ikaros cDNA and the derived amino acid sequence encoded thereby (SEQ ID NO:2).

An Ikaros Protein

The Ikaros protein shown in Fig. 2 (Ik-2) is comprised of 431 amino acids with five CX₂CX₁₂HX₃H zinc finger motifs organized in two separate clusters. (See also Fig. 5.) The first cluster of three fingers is located 59 amino acids from the initiating methionine, while the second cluster is found at the C terminus of the protein 245

amino acids downstream from the first. Two of the finger modules of this protein deviate from the consensus amino acid composition of the Cys-His family of zinc fingers; finger 3 in the first cluster and finger 5 at the C terminus have four amino acids between the histidine residues. This arrangement of zinc fingers in two widely separated regions is reminiscent of that of the *Drosophila* segmentation gap gene Hunchback. Similarity searches in the protein data base revealed a 43% identity between the second finger cluster of Ikaros and Hunchback at the C terminus of these molecules. This similarity at the C terminus of these proteins and the similar arrangement of their finger domains raises the possibility that these proteins are evolutionary related and belong to a subfamily of zinc finger proteins conserved across species.

Ikaros isoforms

In addition to the cDNA corresponding to Ik-2, four other cDNAs produced by differential splicing at the Ikaros genomic locus were cloned. These isoform encoding cDNAs were identified using a 300 bp fragment from the 3' of the previously characterized Ikaros cDNA (Ik-2, Fig. 1). As shown in Fig. 4 and 5, each isoform is derived from three or more of six exons, referred to as E1/2, E3, E4, E5, E6 and E7. All five cDNAs share exons E1/2 and E7 encoding respectively for the N-53 and C-terminal 236 amino acid domains. These five cDNAs consist of different combinations of exons E3-6 encoding the N-terminal zinc finger domain. The Ik-1 cDNA encodes a 58 kD protein with four zinc fingers at its N-terminus and two at its C-terminus and has the strongest similarity to the *Drosophila* segmentation protein Hunchback (Zinc fingers are indicated as F1, F2+F3, F4, and F5+F6 in Fig. 5). The Ik-2 and Ik-3 cDNAs encode 48kd proteins with overlapping but different combinations of zinc fingers. The IK-3 contains fingers 1, 2, 3 while Ik-2 contains fingers 2, 3 and 4. The 43.5 kD Ik-4 protein has two fingers at its N-terminus also present in Ik-1 and Ik-2. The Ik-5 cDNA encodes a 42kd protein with only one N-terminal finger shared by Ik-1 and Ik-3 (Fig. 1). This differential usage of the zinc finger modules by the Ikaros proteins support an overlapping but differential DNA binding specificity.

cDNA cloning of isoforms was performed as follows. A cDNA library made from the T cell line EL4 in λ ZAP was screened at high stringency with a 300 bp fragment from the 3' of the previously described Ikaros cDNA (isoform2). Positive clones were characterized by sequencing using an antisense primer from the 5' of exon 7.

Ikaros Expression

Tissue Specific Expression of the Ikaros Gene

The Ikaros gene is expressed in T cells and their progeny. In the adult mouse, Ikaros mRNA is restricted to the thymus and the spleen with expression in the thymus

being about 3 fold higher than the spleen. Spleen cells preparations depleted of T cells expressed very low levels of this message. Examination of Ikaros expression in cell lines confirm the view that the Ikaros gene is expressed in T cells and their progeny. Ikaros mRNA was detected in a number of T lymphoma cell lines. The T cell line EL4 expressed the highest levels while DO11.10, BW5147 and SL12.1 lymphomas showed moderate to low expression. No expression or very low levels were detected in cell lines representing other hemopoietic lineages including the bone marrow derived progenitor cells FDCPI that exhibit myeloid morphology and differentiation potential, the mast cell line RBL, the macrophage line J774 (detected expression is 25 fold lower than that in thymocytes) and MEL cells which were induced to differentiate into erythroid cells. Nevertheless, moderate levels of Ikaros mRNA were detected in the B cell lymphoma A20 and in the proerythro leukemia cell line MEL. Immortalization of these cell lines and their leukemic phenotype may account for aberrant expression of this nuclear factor which does not appear to be expressed at significant levels in normal B cells (spleen T cell depleted population, or in erythroid progenitors in vivo (from in situ data). Alternatively expression of this thymocyte restricted factor in these cell lines may reflect the existence of an early progenitor with the ability to differentiate into the lymphoid or the erythroid lineage.

Tissue distribution of the Ikaros gene was determined by Northern hybridization of total RNAs prepared from: the T lymphoma cell lines EL4, BW5147, DO11.10, SL12.1; the B cell lymphoma A 20; the tissues of thymus, spleen, kidney, brain and heart isolated from an adult mouse; spleen thymocytes (total and polyA-RNA); bone marrow derived stem cell progenitors FDCPI; macrophage cell line J774; mast cell line RBL; undifferentiated MEL and 58hr DMSO induced MEL cells; and finally T depleted spleen cells (TDSC). A 320bp fragment (bp 1230-1550) from the 3' end of the Ikaros Ik-2 cDNA was used as a probe.

Temporal Regulation of the Expression of the Ikaros Gene

To determine when in hemopoiesis the Ikaros gene becomes activated its expression was studied in situ in the developing mouse embryo. Hemopoiesis begins at day seven in the yolk sac of the mouse embryo with the generation of a large population of primitive erythroblasts. The Ikaros mRNA is not detected in the yolk sac at day 8 in contrast to the erythroid specific transcription factor GATA-1 which is expressed at this time in development. In the embryo proper, expression of Ikaros is first detected in the early liver rudiment at the onset of its hemopoietic function (day-9 1/2 - 10 1/2). At this time, pluripotent stem cells as well as more restricted progenitors are found in the liver which can successfully reconstitute irradiated animals with the whole spectrum of hemopoietic lineages. Expression of the Ikaros gene remains strong in the liver up to day fourteen and begins to decline thereafter although the liver is the major site of hemopoiesis through mid gestation and remains

active through birth. The declining expression of the Ikaros gene in the fetal liver at mid gestation is consistent with changes in the hemopoietic profiles from pluripotent stem cells to more committed erythroid progenitors.

The second site of Ikaros expression is in the thymic rudiment around day 12 when lymphopoietic stem cells are first colonizing this organ. A group of expressing cells is detected at the center of the thymic rudiment surrounded by non expressing cells in the periphery. Expression in the developing thymus becomes quite prominent by day 16 and persists throughout embryogenesis to the adult organism. At these developmental stages expression of Ikaros mRNA is detected throughout the thymus with levels in the medulla sections being slightly more elevated than these in the cortex.

Ikaros expression is first detected in the spleen during late gestation at low levels compared to those of the thymus (day 19). Although the spleen is active in erythropoiesis and myelopoiesis from mid-gestation, its population with mature T cells from the thymus takes place late in embryogenesis and correlates with the late expression of the Ikaros gene. No expression of Ikaros message is detected in the bone marrow of the long bones or the spinal column at day 19 in contrast to the myeloid specific factor Spyl and to the erythroid factor GATA-1. The pattern of expression of the Ikaros gene detected in distinct hemopoietic sites throughout embryonic development is consistent with its restriction to T cells and their progenitors. The only other site in the mouse embryo that exhibited Ikaros expression was a restricted area in the brain which gives rise to the proximal corpus striatum (day 12 through 19).

Embryos were harvested from time pregnant CD1 mice (Charles River) and were fixed in 4% paraformaldehyde for 2 hours to 2 days depending on size. A series of dehydration steps was performed in alcohols followed by xylenes before paraplast embedding. Sections were prepared and treated according to published protocols. Sense and antisense P-UTP RNA probes 300 bp in size were made from the 3' untranslated region of the Ikaros cDNA and were used to hybridize to selected slides at 48°C overnight. After high stringency washings slides were dehydrated and dipped in diluted photographic emulsion (NBT2) for 3 weeks. Dipped slides were developed, stained with Giemsa and analyzed by bright and dark field microscopy.

Expression of Ikaros Isoforms

The pattern of Ikaros isoforms expression in the developing embryo was studied. Two sets of primers were used to amplify the five cDNAs as distinct sized bands from embryonic and postnatal tissues (Fig. 6). A third set of primers complementary to the β -actin cDNA was used to normalize the amount of cDNA used in the reaction. Primers 1/2 amplified a 720, a 457 and a 335 bp fragment from the Ik-1, Ik-2 and Ik-4 cDNAs. Primers 3/4 amplified a 715, a 458 and a 293 bp fragment

from the Ik-1, Ik-3 and Ik-5 cDNAs. A 650bp band detected is an artifact of Ik-1 and Ik-2 coamplification representing Ik-1/Ik-2 and Ik-1/Ik-3 hybrid molecules. It is present at significant levels at the later amplification cycles when the primers to Ik-1, Ik-2 and Ik-3 ratio is decreased. This band is also detected when we coamplify Ik-1, Ik-2 and Ik-3 DNA templates. The identity of the above described bands were also confirmed by cloning and sequencing. It is noteworthy that the 650 bp species was never cloned as a novel of cDNA.

During embryonic development all five Ikaros mRNAs were expressed in hemopoietic centers and in the brain at relatively different levels. The Ik-1 mRNA was abundantly expressed in the early fetal liver and in the maturing thymus while Ik-2 was second in the relative concentration. The Ik-4 isoform was expressed at low levels compared to Ik-1 and Ik-2 in the early fetal liver and in the maturing thymus (liver E14, thymus E16 and D1). However it was expressed at comparable amounts to Ik-1 and Ik-2 in the early thymus and mid-gestation liver (Table 1, thymus E14, liver E16). The Ik-3 and Ik-5 isoforms were expressed but at significantly lower levels than Ik-1 and Ik-2 throughout development (Table 1). All five isoforms were expressed in the embryonic brain. The Ik-1 was the most abundant mRNA, Ik-2 and Ik-4 were present at similar but lower levels while Ik-3 and Ik-5 were the least expressed.

The expression pattern of the Ikaros isoforms detected in the late embryonic thymus persisted past birth while the declining liver expression was switched off. The neonatal spleen expressed only Ik-1 and Ik-2 mRNAs at significant amounts. Low concentration of Ik-1 were still detected in the neonatal brain. These data agree and further supplement our previous in situ hybridization studies performed using an RNA probe made from the 3' of the Ikaros gene shared by all identified Ikaros splicing products.

Table 1. A summary of the embryonic expression patterns for the Ik-1-5 transcripts.

		Ik-1	Ik-2	Ik-3	Ik-4	Ik-5
Liver	E14	+++++	++++	++	++/-	-
	E16	+++	++	+/-	++/-	-
	D1	+	-	-	-	-
Thymus	E14	+++	+++	+/-	+++	-
	E16	++++	+++	+/-	+	+/-
	D1	+++	+++	-	+	+
Brain	E14	++	+	+/-	+	+
	E16	++	+	+/-	+	+
	D1	+	-	-	-	-
Spleen	D1	+++	+++	-	-	-

Embryonic tissues were obtained from embryos of time pregnant mothers. 2 μ g of total RNA prepared from the thymus, liver, brain and spleen at different stages of embryonic development were used for cDNA synthesis with random hexamers and Superscript RNaseH. 1/10th of cDNA made was used in PCR amplification with the 1/20, 3/4 and actin A/B set of primers. PCR reactions were denatured at 95°C for 5 minutes, polymerase was added at 80°C, and then were amplified for 25 cycles at 94°C for 45", 63°C for 1' and 72°C for 1'. PCR amplification for the actin cDNAs were performed for 30 cycles. Products were separated on 2% Seakam FMC agarose, bands were excised, cloned (TA cloning kit, Clontek) and sequenced to verify their identity.

Ikaros stimulates the transcription from the δ A element

Initial Transcriptional Studies

We examined whether the Ikaros protein that can bind to the δ A element can also activate transcription from this binding site. The tkCAT reporter gene under the control of either a reiterated δ A binding site (+/-CRE/-G) or under the control of the CD3 δ enhancer was cotransfected with a recombinant vector expressing the Ikaros gene in the kidney epithelial cell line CV1. Expression of the Ikaros gene in non T cells strongly stimulated transcription from the G box of a reiterated δ A element and in the context of the CD3 δ enhancer (see Fig. 1C). Activity of the δ A and δ Amu1(-CRE) elements was stimulated by eight and seven fold respectively while expression of the CD3 δ enhancer was stimulated by five fold. Since the CD3 δ enhancer is comprised of at least two regulatory elements, expression of all the transcription factors that bind to these sites is necessary for its full activation potential. Expression of the Ikaros gene did not significantly stimulate the activity of the thymidine kinase promoter or of the δ Amu2(-Gbox) element (see Fig. 1C). These data confirms our hypothesis that the Ikaros gene can control activity of the T cell specific δ A element of the CD3 δ enhancer and suggests that it can mediate expression of at least the CD3 δ gene in T cells.

The expression pattern of the Ikaros protein, and its ability to modulate the activity of the CD3 δ enhancer, is consistent with a role in mediating gene expression in T cells in the embryo and in the adult. Its early expression in fetal liver hemopoietic stem cells suggests that it may be expressed in early prothymocyte progenitors and raiser the possibility that it is responsible for commitment of a pluripotent stem cell to the T cell lineage.

Binding Site Selections for the Ikaros 1-3 Isoforms

To investigate the possibility that differential usage of zing finger modules at the N-terminus of the five Ikaros isoforms contributes to their DNA binding a specificity we cloned high affinity binding sites for three of these proteins. The Ik-1, Ik-2 and Ik-3 proteins were selected since they contain either all four (Ik-1) or two

distinct combination of three (Ik-2 and Ik-3) from the pool of the N-terminal four fingers (Fig. 5). We expected these proteins to overlap in specificity with Ik-4 and Ik-5 proteins which contain only two or one of these putative DNA binding modules.

After five rounds of binding site selections from a pool of random oligonucleotides the Ik-1-, Ik-2- and Ik-3- selected oligomers were cloned, sequenced and aligned to a shared motif (Tables 2, 3 and 4 in the tables, bold face type indicates conserved sequence).

Table 2

Ik1-1	aggcgaTTTTG GGGA ATTT C acacc	(SEQ ID NO: 9)
Ik1-2	aggcCATGGGAATGAAGGA A acacc	(SEQ ID NO: 10)
Ik1-3	ggtgtAAATTGGGAATGCTG T gcct	(SEQ ID NO: 11)
Ik1-4	aggcATGGGAATGTCT TGGA acacc	(SEQ ID NO: 12)
Ik1-5	aggcATTAAATGGGAATA A acacc	(SEQ ID NO: 13)
Ik1-6	ggtgtAGGAATGCGGTAATT gcct	(SEQ ID NO: 14)
Ik1-7	ggtgtGGGAATAACTGGGAT gcct	(SEQ ID NO: 15)
Ik1-8	ggtgtGGGAATGTCACTT C acacc	(SEQ ID NO: 16)
Ik1-9	ggtgtGGGAATACTGAGTATGCCT gcct	(SEQ ID NO: 17)
Ik1-10	aggcAAATTTGGGAATA C acacc	(SEQ ID NO: 18)
Ik1-11	ggtgtGTGGGAACATGGGAT gcct	(SEQ ID NO: 19)
Ik1-12	aggcCTATTTCCCTTGGGA A acacc	(SEQ ID NO: 20)
Ik1-13	ggtgtGGAACATCGTGGGAAGCC gcct	(SEQ ID NO: 21)
Ik1-14	aggcGCTTGGGAAAT TCCA acacc	(SEQ ID NO: 22)
Ik1-15	aggcATTCTTAAACCGGGA A acacc	(SEQ ID NO: 23)
Ik1-16	aggcACAAT TCC TT CGGGA acacc	(SEQ ID NO: 24)
Ik1-17	ggtgtCGGGCTTCGGGAAT gcct	(SEQ ID NO: 25)
Ik1-18	gtgt TCC AAACTCGGGAAT gcct	(SEQ ID NO: 26)
Ik1-19	ggtgtGGAATCGGGAATTT gcct	(SEQ ID NO: 27)
Ik1-20	aggcTTATCGGGAAA ACTT acacc	(SEQ ID NO: 28)
Ik1-21	gtgt TCC AAACGGGGGAAT gcct	(SEQ ID NO: 29)
Ik1-22	ggtgtGCAAT TCCA AGGAAT gcct	(SEQ ID NO: 30)
Ik1-23	aggcGCCAT TCCA AGGATA A acacc	(SEQ ID NO: 31)
Ik1-24	aggcTAATCTTGGAAT TCC acacc	(SEQ ID NO: 32)
Ik1-25	gtgtGGACCAT TGGG ATGC gcct	(SEQ ID NO: 33)
Ik1-26	ggtgt TCCA AGAATCAGGAT gcct	(SEQ ID NO: 34)

	A	N	T	T	G	G	G	A	A	T	A/G	C/T	C/T	(SEQ ID NO: 35)
	-3	-1	-2	1	2	3	4	5	6	7	8	9	10	
G	1	0	3	0	21	24	26	3	0	1	6	1	2	
A	8	6	5	1	2	2	0	23	26	2	8	3	2	
T	5	6	10	17	2	0	0	0	0	19	4	6	4	
C	4	7	4	7	1	0	0	0	0	4	1	8	7	

Table 3

IK2-1	ggtgtACGGTTGGGAATGCGgcct	(SEQ ID NO:36)
IK2-2	ggtgtAGGAATGGGAATACAgcct	(SEQ ID NO:37)
IK2-3	ggtgtTGGGATTGGGAATGTgcct	(SEQ ID NO:38)
IK2-4	ggtgtCGGGAATTATTTTAGgcct	(SEQ ID NO:39)
IK2-5	ggtgtAAAAATGGGAACAAAgcct	(SEQ ID NO:40)
IK2-6	ggtgtGGGAAAGATATAGCCgcct	(SEQ ID NO:41)
IK2-7	ggtgtTTAACCAATTGGGAAgcct	(SEQ ID NO:42)
IK2-8	ggtgtTCCGGTATTTGGGAAgcct	(SEQ ID NO:43)
IK2-9	ggtgtGGGATAACTTGGGAAgcct	(SEQ ID NO:44)
IK2-10	aggcGGGAAAACCCATAGGacacc	(SEQ ID NO:45)
IK2-11	ggtAATCCGTCGGGAACAgcctA	(SEQ ID NO:46)
IK2-12	ggcTTTAGATCAGGGAAcacc	(SEQ ID NO:47)
IK2-13	gtATCCTGGTAGGAATCgcct	(SEQ ID NO:48)
IK2-14	aggcTATCCCAGGAATTTGacacc	(SEQ ID NO:49)
IK2-15	aggcAAATTGTTcAGGAACAacacc	(SEQ ID NO:50)
IK2-16	ggtgtCCATAAGGAACAATAgcct	(SEQ ID NO:51)
IK2-17	aggcAGACCCAAGGAAGCCacacc	(SEQ ID NO:52)
IK2-18	aggcTATCCCAGGAATTTGacacc	(SEQ ID NO:53)
IK2-19	aggAGAATCCTATGGGATacacc	(SEQ ID NO:54)
IK2-20	ggtgtTCATTGGGATAGCATgcct	(SEQ ID NO:55)
IK2-21	ggtgtTGGGATTCTGGATAgcct	(SEQ ID NO:56)
IK2-22	aggcGTTTGGGATGTATTTacacc	(SEQ ID NO:57)
IK2-23	ggtgtGGGATCGCCATATTC	(SEQ ID NO:58)
IK2-24	ggtgtGGGATTGCTTTATTT	(SEQ ID NO:59)
IK2-25	ggtgtGGGATTGGGACTAAAgccta	(SEQ ID NO:60)
IK2-26	ggtgtGGGATTGGGACTAAAgcct	(SEQ ID NO:61)
IK2-27	ggtgtAAGGACAATGGGATAgcct	(SEQ ID NO:62)
IK2-28	ggtgtCAGGATTTGGGACACgcct	(SEQ ID NO:63)

IK2-29	ggtgtGGGACTCAAAGAGGC	(SEQ ID NO:64)
IK2-30	ggtgtCCTCCAGCGGGATAAgcct	(SEQ ID NO:65)
IK2-31	aggcATCCGGGATAATAAAacacc	(SEQ ID NO:66)
IK2-32	ggtgtTCTTCGGGATGGCTTgcct	(SEQ ID NO:67)
IK2-33	aggcTTCACCGGGAGCACGacacc	(SEQ ID NO:68)
IK2-34	ggtgtAGATCCCAGGGATTTgcct	(SEQ ID NO:69)
IK2-35	ggtgtAGGTAGGGACATCCCgcct	(SEQ ID NO:70)
IK2-36	ggtgtGAGAAATAAGGGATAgcct	(SEQ ID NO:71)
IK2-37	aggcAAAAAAAAGGGGATAacacc	(SEQ ID NO:72)
IK2-38	gtgtGAAATCTGAGGATCTgcct	(SEQ ID NO:73)

	N	N	T	T	G	G	G	A	A/T	N	N	C	(SEQ ID NO:74)
	-3	-1	-2	1	2	3	4	5	6	7	8	9	
G	6	4	2	2	31	38	38	0	1	3	8	4	
A	9	11	6	6	7	0	0	38	18	10	10	7	
T	4	9	15	20	0	0	0	0	15	13	8	4	
C	10	6	7	10	0	0	0	0	4	8	3	12	

Table 4

IK3-1	AGGCTGGGAATACCAgacacc	(SEQ ID NO:75)
IK3-2	aggcTTGGGATTGGGAATAacacc	(SEQ ID NO:76)
IK3-3	ggtgtTTCCTGGGAATGTTCCgccta	(SEQ ID NO:77)
IK3-4	aggcGTGGGAATATCAGGacacc	(SEQ ID NO:78)
IK3-5	aggcTGGGAATGCTGGGAAacacc	(SEQ ID NO:79)
IK3-6	ggtgtTTGGGAATGCTGGAATgccta	(SEQ ID NO:80)
IK3-7	ggtgtTAATTGGGAATTTTTAgccta	(SEQ ID NO:81)
IK3-8	ggtgtTGGGAAAAGTGGGAATgccta	(SEQ ID NO:82)
IK3-9	ggtgtTTCCTGGGAATGCCAAgccta	(SEQ ID NO:83)
IK3-10	aggcTACAGAATACTGGGAacacc	(SEQ ID NO:84)
IK3-11	aggcTAAAAATTCCTGGGAacacc	(SEQ ID NO:85)
IK3-12	aggcATTCCCGTTTTGGGAacacc	(SEQ ID NO:86)
IK3-13	aggcATTCCCGTTTTGGGAacacc	(SEQ ID NO:87)
IK3-14	ggtgtTATCCCGGGAATACCGccta	(SEQ ID NO:88)
IK3-15	aggcTAAGGAATACCGGGAacacc	(SEQ ID NO:89)
IK3-16	aggcTCTGGAATATCGGGAacacc	(SEQ ID NO:90)
IK3-17	ggtgtTAAATCGGGAATTCCGccta	(SEQ ID NO:91)

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IK3-18	aggcCGGGAATACCGGAAAcacc	(SEQ ID NO:92)
IK3-19	aggcAAAACATTACAGGGAacacc	(SEQ ID NO:93)
IK3-20	aggcAGGGAATATGGGATacacc	(SEQ ID NO:94)
IK3-21	ggtgTAGGAATTCTAGGAATgccta	(SEQ ID NO:95)
IK3-22	aggcATTCCAAGGAATTTacacc	(SEQ ID NO:96)
IK3-23	ggtgTAAGGAATACTGGAATgccta	(SEQ ID NO:97)
IK3-24	ggcAGAATTCCAAGGAATacacc	(SEQ ID NO:98)
IK3-25	aggcCAAGGAATATCAGGAacacc	(SEQ ID NO:99)

	T	N	A/C or T	T	G	G	G	A	A	T	A/G	C/T	C/T	(SEQ ID NO:100)
	-3	-1	-2	1	2	3	4	5	6	7	8	9	10	
G	1	0	2	0	20	25	25	0	0	0	4	0	0	
A	3	8	0	6	5	0	0	25	25	0	8	0	0	
T	13	3	7	14	0	0	0	0	0	18	4	6	7	
C	0	6	10	5	0	0	0	0	0	7	0	9	8	

Table 5

TCE α enhancer			
	m	TGGAGGGAAGTGGGBAACTTTT	(SEQ ID NO:103)
		TGGAAGTGGGAGGC	(SEQ ID NO:104)
		GAGGAGAAAGGTCTCCTAC	(SEQ ID NO:105)
TCR β enhancer			
	h	AACAGGGAAACA	(SEQ ID NO:106)
	m	GTCAGGGAAACAGG	(SEQ ID NO:107)
	h	AAGGTGGGAAGTAA	(SEQ ID NO:108)
	h	GGTAGGAATBGG	(SEQ ID NO:109)
	m	GGAGGGGGAAGAA	(SEQ ID NO:110)
	m	AGTGGGGAAAABTCT	(SEQ ID NO:111)
	m	GGTCAGGGAAACAA	(SEQ ID NO:112)
	m	TGGGGGAAGGGGTGGAAG	(SEQ ID NO:113)
	m	TTTTGGGAACC	(SEQ ID NO:114)
	m	AAAGGGGAACCC	(SEQ ID NO:115)
	h/m	TGGAGGGAG	(SEQ ID NO:116)
promoter			
	m	AGGGGAAA	(SEQ ID NO:117)

		TTTGGGAATT	(SEQ ID NO:118)
		TGAGAGGAAGAGGAGA	(SEQ ID NO:119)
		CAGGAATT	(SEQ ID NO:120)
TCR- δ enhancer			
	δ E5/m	AAGGAAACCAAAACAGGGGAAG	(SEQ ID NO:121)
	δ E3/m	TTGGAAACCT	(SEQ ID NO:122)
CD3- δ enhancer			
	δ A/h	GTTTCCATGACATCATGAATGGGACT	(SEQ ID NO:123)
	/m	GTTTCCATGATGTCATGAATGGGGGT	(SEQ ID NO:124)
		TTCTTGGGGATTG	(SEQ ID NO:125)
CD3- $\gamma\delta$ promoter			
		GGAGGAACT	(SEQ ID NO:126)
		TTTGGGATG	(SEQ ID NO:127)
		TTCTAGGAAGTAAGGGAATTT	(SEQ ID NO:128)
		GTGGGAAGA	(SEQ ID NO:129)
		TAGGAATTCT	(SEQ ID NO:130)
		TAAGGAAAGG	(SEQ ID NO:131)
		TTTCCAAGTGGGAATC	(SEQ ID NO:132)
CD3- ϵ enhancer			
CD4 promoter			
		TGGGGAAGTT	(SEQ ID NO:133)
CD 11 promoter			
		TTGGGAAGGAT	(SEQ ID NO:134)
		AAGGAACA	(SEQ ID NO:135)
IL2-R α promoter/NFkB			
		CAGGGGAATCTCCCTCTCCAT	(SEQ ID NO:136)
IL2 enhancer			
PuBp		AAGAGGAAAA	(SEQ ID NO:137)
PuBd (NFAT-1)		AGGAGGAAAA	(SEQ ID NO:148)
β -IFN(PRDII)/NFkB			
		GGGAAATTCC	(SEQ ID NO:138)
MHC classII/NFkB			
		GGGGAATCC	(SEQ ID NO:139)
TDT-promoter/LYF			
		TGGGAG	(SEQ ID NO:140)
HIV LTR			

		CAGGGAAGTA	(SEQ ID NO:141)
		CAAGGGACTTTCGCTGGGGACTTT	
		CCAGGGAGGGCG	(SEQ ID NO:142)

A consensus recognition sequence for each of these proteins was derived.

The Ik-1, Ik-2 and Ik-3 core motifs were , respectively;

[a/t/c]-n-[t/a/c]-T-G-G-G-A-A-T-a/g/t-c/t-c/t (SEQ ID NO:6)

n-n-[T/c]-[T/c]-G-G-G-A-A-/T-[t/a/c/]-n-C (SEQ ID NO:7) and

T-[a/c]-[C/T]-G-G-G-A-A-T-[A/t/g]--[C/t]-[C/t])SEQ ID NO:8).

The Ik-1 and Ik-3 sequences shared the seven base pair core T-G-G-G-A-A-T (SEQ ID NO:149). The Ik-3 protein showed strong reference for particular nucleotides both at the 5' and 3' flanking positions of this motif while the Ik-1 protein did not select for any particular bases at these positions. The Ik-2 consensus shared five bases with the Ik-1 and Ik-3 heptanucleotide and exhibited great degeneracy outside this sequence. This may permit for the Ik-2 protein to bind with high affinity to a wider range of recognition sequences. Another feature of the oligonucleotides selected by the Ik-3 protein is that 85% of them contained a second consensus (as underlined in Table 2). In contrast, only 50% and 38% of the oligonucleotides selected by Ik-1 and Ik-2 respectively had the potential for a second binding site (as underlined in Table 1 and 3). This may suggest differences in the affinity of Ik-1, Ik-2 and Ik-3 for the selected core motif. Double recognition sequences may allow for an increase in the apparent binding affinity of these proteins for these sites.

Binding site selections were performed as follows. A pool of random oligomers was designed with 25 base pairs of defined sequence at the 5' an 3' (including BamHI and EcoRI restriction sites) and 15 bases of random sequence in the middle. In the first round of selections Ikaros-GST fusions attached to glutathione agarose beads (20µls bead volume) were used in binding assays together with 500,000 cpm of end labeled random primers. After a 20 minute binding reaction on ice the beads were spun down gently and washed twice to three times with ten fold excess of ice cold 1X binding buffer. Bound primers were eluted in 0.1%SDS 10mMTris pH 7.5 recovered radioactivity was determined and then were phenol extracted and precipitated in the presence of 10µgs of glycogen. 1/5th of recovered DNA was reamplified with primers complementary to the defined 5' and 3' sequences with α -³²P dCTP included in the reaction to generate a homogeneously labeled pool of selected oligomers. All probes were gel purified. In higher rounds decreasing amounts of selected oligomers were used in the binding reactions in order to enrich for higher affinity sites (2000,000/100,000 cpm). Five rounds of selections were performed. At the end of the last round of the eluted DNAs were amplified, digested with EcoRI and BamHI restriction enzymes, cloned in PGEM3Z and sequenced with

normal and reverse primers. Sequences of selected primers were aligned to a shared motif present in all DNAs.

Fusion protein and DNA binding studies were performed as follows. The coding region of the Ikaros isoforms were PCR amplified with Vent polymerase from their respective cDNAs using primers and cloned into the BamHI/EcoRI sites of pGEXIII. Recombinant plasmids were analyzed by sequencing. Overnight cultures of the appropriate recombinant PGEX vectors were diluted by ten fold and grown at 37°C for 90 minutes before a 3 hours induction with 2mM IPTG at 26°C. Crude bacterial lysates were produced as previously described (Georgopoulos 1992). Ikaros-GST fusions were partially purified on glutathione agarose beads, eluted in buffer D containing 20mM free glutathione and 0.5 M NaCl at 4°C for 1 hour. Eluted proteins were checked by SDS-Page their concentrations were estimated by the Lowry method and appropriate dilutions were used for DNA binding studies. DNA binding assays were performed. Binding reactions contained 50,000 cpm of labeled oligonucleotides (0.5- μ g), 100ngs of the fusion proteins, 0.1 μ g of dI/dC and the binding buffer was supplemented with 20 μ M of ZNCl₂. Binding reactions for methylation interference assays were scaled up ten times and were performed as previously described (Georgopoulos 1990).

Binding Specificity of Ikaros Isoform Ik-1-5

The binding specificity of the five Ikaros proteins for a single recognition site derived from the selected consensus was tested in a gel retardation assay. A 24 bp oligonucleotide (1K-BS1-T-t-T-T-G-G-G-A-A-T-A-C-Cc) (SEQ ID NO:101) designed to accommodate high affinity binding of the three selecting proteins was tested. The Ik-1 isoform bound this sequence with the highest affinity followed by Ik-2 and Ik-3. The presence of only two or one zinc fingers at the N-terminus of Ik-5 and Ik-4 were not sufficient for their stable interaction with this site. An oligonucleotide containing a second low affinity site in close proximity to the first one was then tested (IK-BS2)TCAGCTTTTGGGAATCTCCTGTCA (SEQ ID NO:150). Four of the isoforms bound to this sequence and only Ik-5 with the single N-terminal finger did not. We then examined the ability of the short Ik-2 core motif to bind to these proteins (IK-BS3, TCAGCTTTTGGGATTCTCCTGTCA (SEQ ID NO:151)). Ik-2 bound equally well to the five versus the seven base pair core, while Ik-1 bound to this site with at least a three fold lower affinity. The Ik-3 and Ik-4 isoforms did not bind to this sequence. Oligonucleotides with non preferred bases and the 3' and 5' flanking positions of the Ik-1/Ik-3 core bound with high affinity the Ik-2 protein, with lower affinity Ik-1 and did not bind Ik-3 (IK-BS5, TCAGCGGGGGGAATACCCTGTCA (SEQ ID NO:152)). This was in line with the selection data with Ik-2 being the most promiscuous of the three proteins followed by Ik-1 and with Ik-3 being the most restricted in binding specificity.

The Ik-5 protein did not interact with any of these DNAs. However it bound with low affinity but in a sequence specific manner to the δ A element of the CD3 δ enhancer. The same complex was detected for Ik-4 while Ik-1, Ik-2 and Ik-3 bound this DNA in a qualitative and quantitatively different manner. The weak binding of Ik-4 and Ik-5 to the δ A element is probably mediated by the two C-terminal fingers while the high affinity binding of Ik-1, Ik-2 and Ik-3 is mediated by the N-terminal finger domain (also documented by selection and binding studies with the N-terminal finger domains).

These binding data on the five Ikaros isoforms substantiate further the previously described selections and demonstrate that the Ikaros proteins can bind to overlapping but also distinct recognition sequences.

Chemical Footprinting of Ikaros Isoforms Ik-1-4 on their cognate sites.

The protein/DNA interactions of Ik-1-4 were further established by chemical footprinting. The IK-BS2 oligonucleotide (TCAGCTTTTGGGAATCTCCTGTCA) (SEQ ID NO:102) that binds with high affinity to the four isoforms was used in a methylation interference assay. On the positive strand all four proteins made similar contacts. The three guanines at positions 2, 3 and 4 of the consensus interfered 100% with the binding of all four proteins. Ik-2 made additional major groove contacts with the guanine at position -5 and with the adenine at position 5. On the negative strand the four proteins gave dramatically footprints. Ik-2 made contact only with the guanine at position -3 while Ik-1 made an additional partial contact with position -1. The Ik-3 protein made contacts with position 1 and -2 adenines and -1 and -3 guanines. The Ik-4 protein footprint was the most extensive including the three guanines at position -1, -3 and -4 and the adenine at position -5.

Of the three proteins Ik-3 with the strictest binding specificity made the most DNA contacts. The Ik-2 protein the most promiscuous of the three, made the least DNA contacts. Finally the extensive but qualitative different footprint made by Ik-4 further support the cooperative occupancy of close proximity recognition sites by this protein. These methylation interference data demonstrate that the four Ikaros proteins make qualitative distinct DNA contacts and underling their ability to bind DNA differentially.

Transcriptional activation by the IK proteins.

We had previously shown that Ik-2 can activate transcription from a multimerized δ A element to moderate levels. Here we investigate the ability of four of the Ikaros isoforms to activate transcription from a reiterated copy of a preferred binding site in transient expression assays in NTH-3T3 fibroblasts. The IK-BS3 oligonucleotide which binds Ik-1-4 with high affinity was tested for its ability to stimulate transcription of the tkCAT reporter gene upon expression of these proteins.

The activity of this reporter gene detected in this fibroblast cell line was stimulated by 22 fold when cotransfected with an expression vector harboring the Ik-1 cDNA. The activity of this reporter gene was stimulated by the Ik-2 expression vector by 11 fold. However, expression of the Ik-3 and -Ik-4 cDNAs stimulated transcription only by 2 to 3 fold. This suggested that these proteins may function to attenuate transcription from binding sites that also accommodate Ik-1 and Ik-2. (Transient protein expression of the IKaros cDNAs was comparable).

The Ik-1 and Ik-2 enhancer proteins were not able to activate transcription from a mutant binding site which did not bind any of these factors demonstrating that their activation potential is sequence specific.

Since the sequence composition for an Ikaros high affinity binding site is identical to the NFkB motifs present in the IL2-Receptor α and the β -interferon promoters we examined its transcriptional activity in a mature T cell line in the absence and in the presence of mitogenic stimulation. We have chosen the human Jurkat T cell line for the following reasons. First the activity of NFkB recognition sequences that closely match the selected Ikaros binding sites have been extensively studied in this cell line and secondly because we know that the human Ikaros gene is highly conserved to the mouse gene in both amino acid composition and splicing variants. In contrast to previous reports we detected high levels of transcriptional activity from this multimerized site which were not further stimulated upon mitogenic treatment. This activity was decreased by *five* fold when Ikaros antisense expression vectors cotransfected together with this reporter gene. No such effect was detected when reporter genes driven by the RSV or SL3 LTRs were used in a parallel experiment suggesting that transcriptional inhibition by the Ikaros antisense RNA is specific to this site.

Transcriptional activation from a reiterated NFkB variant in NIH3T3 fibroblasts upon expression of Ikaros 1-4 isoforms was determined as follows. The stimulation of CAT activity in the presence of the Ikaros proteins was evaluated as the ratio of activity when cotransfected with a recombinant CDM8/CDM8 vector alone. This data represent an average of three/four experiments with each combination of transfected plasmids per experiment repeated twice. All transfections were normalized to GH levels as described in materials and methods.

Activity and repression of the reiterated NFkB like element in human T cells was determined as follows. The reporter gene under the control of Ik-BS2(FNKB like variant) or of RSV and SL3 LTRs was transfected in Jurat cells in the presence of CDM8 expressing Ikaros antisense plasmids. Fold induction relative to enhancerless plasmid and suppression in presence of antisense RNAs was determined.

Mammalian expression vector and transfection experiments were performed as follows. The five Ikaros isoforms were subcloned into the HindIII-Not I site of the

CDM8 expression vector. The tkCAT reporter gene under the control of four sense copies of IKBS1 IKBS2 IKBS7 and 8 were cotransfected with the appropriate expression vectors and with the SV40 or RSV GH plasmids in the NIH 3T3 fibroblasts and the mature T cell line Jurkat as described previously. Cells were harvested 36-48 hours later and analyzed for CAT activity and Growth hormone levels. Results determined as the average of 3/4 independent experiments where each combination of reporter to expression plasmids was performed twice.

Target sites for the Ikaros proteins in lymphoid restricted regulatory domains.

Potential high affinity binding sites for the Ikaros proteins were found in the enhancer and promoter regions of the TCR- α , - β , and - δ , the CD3 - δ , - ϵ , and - γ genes the SL3 and HIV LTR and in the regulatory domains of other T cell restricted antigens (Table 5). Some of these sites can bind all four proteins while others interact only with Ik-2 and Ik-1. The selected recognition sequences for the three Ikaros isoforms match closely the NFkB motif present in the promoter of the IL2-R α , in the PRDII element of the β - interferon, and in the H-2K gene. This NFkB site binds with high affinity four of the IKaros proteins.

Related sequences to the Ikaros motif were also found in the above described regulatory domains as well as to the Ikaros motif were also found in the above described regulatory domains as well as in the purine boxes of the IL2 gene in the LYF site of the TDT promoter as well as in the NFkB variant sites of the HIV LTR (Table 5). To investigate the affinity of the Ikaros proteins for these sites we studied their ability to compete with the selected recognition sequences. Base pair substitutions within and outside the seven base pair motif were introduced to match the sequence composition of some of these sites present in the lymphoid and T cell specific regulatory domains. Oligonucleotides with the appropriate base pair changes were used in competition experiments against the consensus motif (IKB-S1).

The Ik-BS2 oligonucleotide, identical to the IL2-R α NFkB motif, bound to the four proteins with a two fold higher affinity than a single copy of the consensus motif. We believe that this is due to the second low affinity binding site in the opposite strand.

The existence of low affinity binding sites in close proximity in a regulatory domain increases the relative affinity of the Ikaros proteins for these sites. This is clearly the case with δA and possibly with other elements. The occupancy of high affinity binding sites could also be affected by low affinity sites in the immediate region. The apparent binding constant of these proteins for these sites may raise to an even higher value and could dictate the order of target genes activated by the Ikaros enhancers in the developing lymphocyte.

Cloning of the human Ikaros gene

Since the cDNA sequence of the mouse Ikaros gene has been determined the human Ikaros gene can be cloned using any one of a number of techniques known to those skilled in the art. Such cloning techniques are described in detail in (Molecular Cloning: A Laboratory Manual, Sambrook et al. 1989, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, hereby incorporated by reference). For example, oligonucleotides corresponding to stretches of 1020 amino acid residues of the coding region of mouse Ikaros can be synthesized on an oligonucleotide synthesizer. These oligonucleotides can be used as probes to screen a human cDNA library, e.g., a library from Jurkat T cells, or a human genomic library. Positive clones are sequenced, the sequence is compared to the known amino acid sequence of mouse Ikaros. Functional Ikaros activity encoded by these DNAs can be tested as described herein.

The partial sequence of an Ikaros human cDNA is shown in Fig. 3 (SEQ ID NO:3).

The Ikaros genomic locus

Based on sequence analysis of variant cDNAs, the genomic locus is thought to include about 9-11 exons. Genomic DNAs encompassing most or all of the Ikaros exons present in the genome were isolated by screening a mouse genomic SV129 library made into the λ DASH II phage vector using the various Ikaros cDNAs as probes. The Ikaros gene includes at least 80-90kb of genomic sequence which was isolated as distinct but also overlapping genomic clones. Some of the Ikaros genomic clones are indicated in Figs. 7. The exons are depicted as boxes while the introns as lines. The DNA sequence for: the 5' boundary (SEQ ID NO:143) and the 3' boundary (SEQ ID NO:144) of exon E5; the 5' boundary (SEQ ID NO:145) of exon E3; and the 5' boundary (SEQ ID NO:146) and the 3' boundary (SEQ ID NO:147) of exon E7, were determined.

Homologous recombination experiments in vitro and in vivo and knockout mice.

To address the role of the lymphoid restricted transcription factor Ikaros in vivo we targeted mutations at the mouse Ikaros genomic locus in embryonic stem cells (E.S). Two targeting vectors carrying distinct deletions at the Ikaros genomic locus were transfected in the J1 E.S line derived from the SV129 mouse (En li, Cell 1992). Homologous recombination events in the E.S cells were scored by a double selection counter selection scheme; G418 and FIAU were used in the media to select for neomycin gene activity and for the absence of thymidine kinase gene activity. The neo gene is located in the middle of the construct while the tk gene is present at the 5' or 3' of the targeting vector and allows for selecting against non-homologous recombination events. E.S. cell lines carrying either mutation one or two were established by Southern analysis and were injected in the blastocysts of Balbe or C57 black mice. The chimeric blastocysts were reimplanted in pseudopregnant mice and

gave rise to chimeric animals. Mice which were more than 70% chimeric for the SV129 strain as determined by coat color (agouti vs white or black background) were bred further. Germ line transmission was determined by coat color (agouti) and by Southern analysis of tail DNA. We are in the process of breeding these mice to obtain animals which are homozygous for these mutations.

Both of the targeted mutations are deletions. The first mutation deletes the last exon, E7, which is shared by all the Ikaros isoforms. This should generate proteins which can bind DNA but which cannot activate transcription. These proteins may function as dominant negative regulators of transcription since they can compete for DNA binding with wild type Ikaros proteins but cannot activate transcription. Mice heterozygous for this mutation may exhibit a decrease in the level of expression of genes that rely on the Ikaros proteins for their regulation. These mice may exhibit a less severe phenotype than the ones with total lack of expression of Ikaros proteins. Analysis of these animals may prove to be necessary if the phenotype on mice with total loss of function is severe.

The second mutation (a deletion of exon E3 and E4) should result in a total loss of function of the Ikaros gene. Mice homozygous for this mutation may have a severe impairment of the Ikaros gene. Mice homozygous for this mutation may have a severe impairment of their immune system as a result of altered expression of genes regulated by the Ikaros gene. Possible candidates for Ikaros regulation are TDT (recombination pathway) CD3 complex. TCR complex IL2 gene HIV LTR etc. Lymphoid cell lines derived from these mice can be used to delineate the regulatory pathway that leads to mature T and B cells but the mice themselves can be used to study the complex interaction between the different lineages in the hemopoietic pathway and design in vivo experiments to study and correct immunodeficiency syndromes. Finally, ES cell lines derived from these animals can be studied by in vivo differentiation into the hemopoietic/lymphopoietic lineage.

Use

The peptides of the invention may be administered to a mammal, particularly a human, in one of the traditional modes (e.g., orally, parenterally, transdermally, or transmucosally), in a sustained release formulation using a biodegradable biocompatible polymer, or by on-site delivery using micelles, gels and liposomes or by transgenic modes.

Other Embodiments

Nucleic acid encoding all or part of the Ikaros gene can be used to transform cells. For example, the Ikaros gene, e.g., a mis-expressing or mutant form of the Ikaros gene, e.g., a deletion, or DNA encoding an Ikaros protein can be used to transform a cell and to produce a cell in which the cell's genomic Ikaros gene has been

replaced by the transformed gene, producing, e.g., a cell deleted for the Ikaros gene. As described above, this approach can be used with cells capable of being grown in culture, e.g., cultured stem cells, to investigate the function of the Ikaros gene.

Analogously, nucleic acid encoding all or part of the Ikaros gene, e.g., a mis-expressing or mutant form of the gene, e.g., a deletion, can be used to transform a cell which subsequently gives rise to a transgenic animal. This approach can be used to create, e.g., a transgenic animal in which the Ikaros gene is, e.g., inactivated, e.g., by a deletion. Homozygous transgenic animals can be made by crosses between the offspring of a founder transgenic animal. Cell or tissue cultures can be derived from a transgenic animal. A subject at risk for a disorder characterized by an abnormality in T cell development or function, e.g., leukemia, can be detected by comparing the structure of the subject's Ikaros gene with the structure of a wild type Ikaros gene. Departure from the wild type structure by, e.g., frameshifts, critical point mutations, deletions, insertions, or translocations, are indicative of risk. The DNA sequence of the coding region of several exons as well as several intron exon boundaries are included herein. Other regions can be obtained or sequenced by methods known to those skilled in the art.

The invention includes any protein which is substantially homologous to an Ikaros protein, e.g., the Ikaros protein shown in SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:5, or other isoforms. Also included are: allelic variations; natural mutants; induced mutants, e.g., in vitro deletions; proteins encoded by DNA that hybridizes under high or low (e.g., washing at 2xSSC at 40 C with a probe length of at least 40 nucleotides) stringency conditions to a nucleic acid naturally occurring (for other definitions of high and low stringency see Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989, 6.3.1 - 6.3.6, hereby incorporated by reference); and polypeptides or proteins specifically bound by antisera to an Ikaros protein, especially by antisera to the active site or binding domain of an Ikaros protein. The term also includes chimeric polypeptides that include an Ikaros protein.

DNA and peptide sequences of the invention can be, e.g., mouse, primate, e.g., human, or non-naturally occurring sequences.

The invention also includes any biologically active fragment or analog of an Ikaros protein. By "biologically active" is meant possessing any *in vivo* or *in vitro* activity which is characteristic of an Ikaros isoform, e.g., an isoform shown in (SEQ ID NO:2) or Fig. 3 (SEQ ID NO:3) or (SEQ ID NO:5), e.g., Ikaros activity as described above. Because the Ikaros proteins exhibit a range of physiological properties and because such properties may be attributable to different portions of the Ikaros protein molecule, a useful Ikaros protein fragment or Ikaros protein analog is one which exhibits a biological activity in any one (or more) of a variety of the Ikaros protein assays, for example, the ability to bind to or stimulate transcription from a δA

element or an NKFB element, as described above. An Ikaros protein fragment or analog possesses, most preferably 90%, preferably 40%, or at least 10%, of the activity of a naturally occurring Ikaros isoform, e.g., of the Ikaros protein shown in (SEQ ID NO:2), (SEQ ID NO:3) or (SEQ ID NO:5), in any *in vivo* or *in vitro* Ikaros assay.

Preferred analogs include Ikaros peptides or exons (or biologically active fragments thereof) whose sequences differ from the wild-type sequence by one or more conservative amino acid substitutions or by one or more non-conservative amino acid substitutions, deletions, or insertions which do not abolish biological activity. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics, e.g., substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Other conservative substitutions can be taken from the table below.

CONSERVATIVE AMINO ACID REPLACEMENTS

For Amino Acid	Code	Replace with any of
Alanine	A	D-Ala, Gly, beta-Ala, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, β -Ala Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Leu, D-Leu, Met, D-Met

Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, cis-3,4, or 5-phenylproline
Proline	P	D-Pro, L-I-thioazolidine-4-carboxylic acid, D-or L-1-oxazolidine-4-carboxylic acid
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Ile, Ile, D-Ile, Met, D-Met

Other useful modifications include those which increase peptide stability; such analogs may contain, for example, one or more non-peptide bonds (which replace peptide bonds) or D-amino acids in the peptide sequence.

Analogues can differ from a naturally occurring Ikaros protein in amino acid sequence or can be modified in ways that do not affect sequence, or both. Analogues of the invention will generally exhibit at least 70% more preferably 80%, more preferably 90%, and most preferably 95% or even, 99%, homology with a segment of 20 amino acid residues, preferably more than 40 amino acid residues or more preferably the entire sequence of naturally occurring Ikaros protein sequence.

Alterations in primary sequence include genetic variations, both natural and induced. Also included are analogues that include residues other than naturally occurring L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids. Alternatively, increased stability may be conferred by cyclizing the peptide molecule.

Nonsequence modification include *in vivo* or *in vitro* chemical derivatization or polypeptides, e.g., acetylation, methylation, phosphorylation, carboxylation, or glycosylation; glycosylation can be modified, e.g., by modifying the glycosylation

patterns of a polypeptide during its synthesis and processing or in further processing steps, e.g., by exposing the polypeptide to glycosylation-affecting enzymes derived from cells that normally provide such processing, e.g., mammalian glycosylation enzymes; phosphorylation can be modified by exposing the polypeptide to phosphorylation-altering enzymes, e.g., kinases or phosphatases.

In addition to substantially full-length polypeptides, the invention also includes biologically active fragments of the polypeptides. As used herein, the term "fragment", as applied to a polypeptide, will be of a length described for an Ikaros peptide above and will ordinarily be at least about 20 residues, more typically at least about 40 residues, preferably at least about 60 residues in length.

Fragments of Ikaros peptides or introns can be made by methods known to those skilled in the art, e.g., by expressing Ikaros DNA which has been manipulated in vitro to encode the desired fragment; e.g., by restriction digestion of an Ikaros DNA e.g., the sequence in SEQ ID NO:1 or SEQ ID NO:2. Analogs can be made by methods known to those skilled in the art, e.g., by in vitro DNA sequence modifications of the sequence of an Ikaros DNA e.g., the sequence in SEQ ID NO:1 or SEQ ID NO:2. For example, in vitro mutagenesis can be used to convert the DNA sequence of SEQ ID NO:1 into a sequence which encodes an analog in which one or more amino acid residues has undergone a replacement, e.g., a conservative replacement as described in the table of conservative amino acid substitutions provided herein. Fragments or analogs can be tested by methods known to those skilled in the art for the presence of Ikaros activity.

Also included are Ikaros protein polypeptides containing residues that are not required for biological activity of the peptide, such as residues that are not required for the biological activity of the polypeptide, or that result from alternative mRNA splicing or alternative protein processing events.

The invention also includes nucleic acids encoding the polypeptides of the invention.

In order to obtain an Ikaros protein one can insert Ikaros-encoding DNA into an expression vector, introduce the vector into a cell suitable for expression of the desired protein, and recover and purify the desired protein by prior art methods. Antibodies to Ikaros proteins can be made by immunizing an animal, e.g., a rabbit or mouse, and recovering anti-Ikaros antibodies by prior art methods.

To obtain a specific splicing-product (i.e., a specific isoform) one can make a synthetic structural gene including only the exons which code for the desired splicing product and express the gene as described above.

Other embodiments are within the following claims.

What is claimed is:

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: The General Hospital Corporation
(B) STREET: 55 Fruit Street
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 02114
(G) TELEPHONE: (617) 726-8608
(H) TELEFAX: (617) 726-1668

(ii) TITLE OF INVENTION: IKAROS: A T CELL PATHWAY REGULATORY GENE

(iii) NUMBER OF SEQUENCES: 152

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: ASCII

(v) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 946,233
(B) FILING DATE: 14-SEP-1992

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (617)227-7400
(B) TELEFAX: (617)227-5941

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGAAGTTTCC ATGACATCAT GAATGGGGGT GGCAGAGA

38

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1788 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 223..1515

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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AATTCGTTCT ACCTTCTCTG AACCCAGTG GTGTGTCAAG GCCGGACTGG GAGCTTGGGG      60
GAAGAGGAAG AGGAAGAGGA ATCTGCGGCT CATCCAGGGA TCAGGGTCCT TCCCAAGTGG      120
CCACTCAGAG GGGACTCAGA GCAAGTCTAG ATTTGTGTGG CAGAGAGAGA CAGCTCTCGT      180
TTGGCCTTGG GGAGGCACAA GTCTGTTGAT AACCTGAAGA CA                          222

ATG GAT GTC GAT GAG GGT CAA GAC ATG TCC CAA GTT TCA GGA AAG GAG      270
Met Asp Val Asp Glu Gly Gln Asp Met Ser Gln Val Ser Gly Lys Glu
1          5          10          15

AGC CCC CCA GTC AGT GAC ACT CCA GAT GAA GGG GAT GAG CCC ATG CCT      318
Ser Pro Pro Val Ser Asp Thr Pro Asp Glu Gly Asp Glu Pro Met Pro
          20          25          30

GTC CCT GAG GAC CTG TCC ACT ACC TCT GGA GCA CAG CAG AAC TCC AAG      366
Val Pro Glu Asp Leu Ser Thr Thr Ser Gly Ala Gln Gln Asn Ser Lys
          35          40          45

AGT GAT CGA GGC ATG GGT GAA CGG CCT TTC CAG TGC AAC CAG TCT GGG      414
Ser Asp Arg Gly Met Gly Gln Arg Pro Phe Gln Cys Asn Gln Ser Gly
          50          55          60

GCC TCC TTT ACC CAG AAA GGC AAC CTC CTG CGG CAC ATC AAG CTG CAC      462
Ala Ser Phe Thr Gln Lys Gly Asn Leu Leu Arg His Ile Lys Leu His
          65          70          75          80

TCG GGT GAG AAG CCC TTC AAA TGC CAT CTT TGC AAC TAT GCC TGC CGC      510
Ser Gly Glu Lys Pro Phe Lys Cys His Leu Cys Asn Tyr Ala Cys Arg
          85          90          95

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CGG AGG GAC GCC CTC ACC GGC CAC CTG AGG ACG CAC TCC GTT GGT AAG	558
Arg Arg Asp Ala Leu Thr Gly His Leu Arg Thr His Ser Val Gly Lys	
100 105 110	
CCT CAC AAA TGT GGA TAT TGT GGC CGG AGC TAT AAA CAG CGA AGC TCT	606
Pro His Lys Cys Gly Tyr Cys Gly Arg Ser Tyr Lys Gln Arg Ser Ser	
115 120 125	
TTA GAG GAG CAT AAA GAG CGA TGC CAC AAC TAC TTG GAA AGC ATG GGC	654
Leu Glu Glu His Lys Glu Arg Cys His Asn Tyr Leu Glu Ser Met Gly	
130 135 140	
CTT CCG GGC GTG TGC CCA GTC ATT AAG GAA GAA ACT AAC CAC AAC GAG	702
Leu Pro Gly Val Cys Pro Val Ile Lys Glu Glu Thr Asn His Asn Glu	
145 150 155 160	
ATG GCA GAA GAC CTG TGC AAG ATA GGA GCA GAG AGG TCC CTT GTC CTG	750
Met Ala Glu Asp Leu Cys Lys Ile Gly Ala Glu Arg Ser Leu Val Leu	
165 170 175	
GAC AGG CTG GCA AGC AAT GTC GCC AAA CGT AAG AGC TCT ATG CCT CAG	798
Asp Arg Leu Ala Ser Asn Val Ala Lys Arg Lys Ser Ser Met Pro Gln	
180 185 190	
AAA TTT CTT GGA GAC AAG TGC CTG TCA GAC ATG CCC TAT GAC AGT GCC	846
Lys Phe Leu Gly Asp Lys Cys Leu Ser Asp Met Pro Tyr Asp Ser Ala	
195 200 205	
AAC TAT GAG AAG GAG GAT ATG ATG ACA TCC CAC GTG ATG GAC CAG GCC	894
Asn Tyr Glu Lys Glu Asp Met Met Thr Ser His Val Met Asp Gln Ala	
210 215 220	
ATC AAC AAT GCC ATC AAC TAC CTG GGG GCT GAG TCC CTG CGC CCA TTG	942
Ile Asn Asn Ala Ile Asn Tyr Leu Gly Ala Glu Ser Leu Arg Pro Leu	
225 230 235 240	
GTG CAG ACA CCC CCC GGT AGC TCC GAG GTG GTG CCA GTC ATC AGC TCC	990
Val Gln Thr Pro Pro Gly Ser Ser Glu Val Val Pro Val Ile Ser Ser	
245 250 255	
ATG TAC CAG CTG CAC AAG CCC CCC TCA GAT GGC CCC CCA CGG TCC AAC	1038
Met Tyr Gln Leu His Lys Pro Pro Ser Asp Gly Pro Pro Arg Ser Asn	
260 265 270	
CAT TCA GCA CAG GAC GCC GTG GAT AAC TTG CTG CTG CTG TCC AAG GCC	1086
His Ser Ala Gln Asp Ala Val Asp Asn Leu Leu Leu Leu Ser Lys Ala	
275 280 285	
AAG TCT GTG TCA TCG GAG CGA GAG GCC TCC CCG AGC AAC AGC TGC CAA	1134
Lys Ser Val Ser Ser Glu Arg Glu Ala Ser Pro Ser Asn Ser Cys Gln	
290 295 300	
GAC TCC ACA GAT ACA GAG AGC AAC GCG GAG GAA CAG CGC AGC GGC CTT	1182
Asp Ser Thr Asp Thr Glu Ser Asn Ala Glu Glu Gln Arg Ser Gly Leu	
305 310 315 320	

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ATC TAC CTA ACC AAC CAC ATC AAC CCG CAT GCA CGC AAT GGG CTG GCT      1230
Ile Tyr Leu Thr Asn His Ile Asn Pro His Ala Arg Asn Gly Leu Ala
                      325                      330                      335

CTC AAG GAG GAG CAG CGC GCC TAC GAG GTG CTG AGG GCG GCC TCA GAG      1278
Leu Lys Glu Glu Gln Arg Ala Tyr Glu Val Leu Arg Ala Ala Ser Glu
                      340                      345                      350

AAC TCG CAG GAT GCC TTC CGT GTG GTC AGC ACG AGT GGC GAG CAG CTG      1326
Asn Ser Gln Asp Ala Phe Arg Val Val Ser Thr Ser Gly Glu Gln Leu
                      355                      360                      365

AAG GTG TAC AAG TGC GAA CAC TGC CGC GTG CTC TTC CTG GAT CAC GTC      1374
Lys Val Tyr Lys Cys Glu His Cys Arg Val Leu Phe Leu Asp His Val
                      370                      375                      380

ATG TAT ACC ATT CAC ATG GGC TGC CAT GGC TGC CAT GGC TTT CGG GAT      1422
Met Tyr Thr Ile His Met Gly Cys His Gly Cys His Gly Phe Arg Asp
                      385                      390                      395                      400

CCC TTT GAG TGT AAC ATG TGT GGT TAT CAC AGC CAG GAC AGG TAC GAG      1470
Pro Phe Glu Cys Asn Met Cys Gly Tyr His Ser Gln Asp Arg Tyr Glu
                      405                      410                      415

TTC TCA TCC CAT ATC ACG CGG GGG GAG CAT CGT TAC CAC CTG AGC      1515
Phe Ser Ser His Ile Thr Arg Gly Glu His Arg Tyr His Leu Ser
                      420                      425                      430

TAAACCCAGC CAGGCCCCAC TGAAGCACAA AGATAGCTGG TTATGCCTCC TTCCCGGCAG      1575

CTGGACCCAC AGCGGACAAT GTGGGAGTGG ATTTGCAGGC AGCATTGTGT CTTTATGTT      1635

GGTTGTTTGG CGTTTCATTT GCGTTGGAAG ATAAGTTTTT AATGTTAGTG ACAGGATTGC      1695

ATTGCATCAG CAACATTCAC AACATCCATC CTTCTAGCCA GTTTTGTTC CTGGTAGCTG      1755

AGGTTTCCCG GATATGTGGC TTCCTAACAC TCT      1788

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1611 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1611

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAA TTC CGC CTT TGG GGG CTG ACC GGG AGC CCG GCG CGA TTG CAA AGT	48
Glu Phe Arg Leu Trp Gly Leu Thr Gly Ser Pro Ala Arg Leu Gln Ser	
1 5 10 15	
TTT CGT GCG CGC CCC TCT GGC CCG GAG TTG CCG CTG AGA CGC GCC GCG	96
Phe Arg Ala Arg Pro Ser Gly Pro Glu Leu Arg Leu Arg Arg Ala Ala	
20 25 30	
CGA GCC GGG GGA CTC GGC GAC GGG GCG GGG ACG GGA CGA CGC ACC CTC	144
Arg Ala Gly Gly Leu Gly Asp Gly Ala Gly Thr Gly Arg Arg Thr Leu	
35 40 45	
TCC GTG TCC GCT CTC GCC CTT CTG CCG GCC CCG CTC CCT GTA CCG GAG	192
Ser Val Ser Ala Leu Ala Leu Leu Arg Ala Pro Leu Pro Val Pro Glu	
50 55 60	
CAG CGA TCC GGG AGG CGG CCG AGA GCC AGT AAT GTT AAA GTA GAG ACT	240
Gln Arg Ser Gly Arg Arg Pro Arg Ala Ser Asn Val Lys Val Glu Thr	
65 70 75 80	
CAG AGT GAT GAA GAG AAT GGG CGT GCC TGT GAA ATG AAT GGG GAA GAA	288
Gln Ser Asp Glu Glu Asn Gly Arg Ala Cys Glu Met Asn Gly Glu Glu	
85 90 95	
TGT GCG GAG GAT TTA CGA ATG CTT GAT GCC TCG GGA GAG AAA ATG AAT	336
Cys Ala Glu Asp Leu Arg Met Leu Asp Ala Ser Gly Glu Lys Met Asn	
100 105 110	
GGC TCC CAC AGG GAC CAA GGC AGC TCG GCT TTG TCG GGA GTT GGA GGC	384
Gly Ser His Arg Asp Gln Gly Ser Ser Ala Leu Ser Gly Val Gly Gly	
115 120 125	
ATT CGA CTT CCT AAC GGA AAA CTA AAG TGT GAT ATC TGT GGG ATC ATT	432
Ile Arg Leu Pro Asn Gly Lys Leu Lys Cys Asp Ile Cys Gly Ile Ile	
130 135 140	
TGC ATC GGG CCC AAT GTG CTC ATG GTT CAC AAA AGA AGC CAC ACT GGA	480
Cys Ile Gly Pro Asn Val Leu Met Val His Lys Arg Ser His Thr Gly	
145 150 155 160	
GAA CGG CCC TTC CAG TGC AAT CAG TGC GGG GCC TCA TTC ACC CAG AAG	528
Glu Arg Pro Phe Gln Cys Asn Gln Cys Gly Ala Ser Phe Thr Gln Lys	
165 170 175	
GGC AAC CTG CTC CGG CAC ATC AAG CTG CAT TCC GGG GAG AAG CCC TTC	576
Gly Asn Leu Leu Arg His Ile Lys Leu His Ser Gly Glu Lys Pro Phe	
180 185 190	
AAA TGC CAC CTC TGC AAC TAC GCC TGC CGC CGG AGG GAC GCC CTC ACT	624
Lys Cys His Leu Cys Asn Tyr Ala Cys Arg Arg Arg Asp Ala Leu Thr	
195 200 205	
GGC CAC CTG AGG ACG CAC TCC GTT GGT AAA CCT CAC AAA TGT GGA TAT	672
Gly His Leu Arg Thr His Ser Val Gly Lys Pro His Lys Cys Gly Tyr	
210 215 220	

TGT GGC CGA AGC TAT AAA CAG CGA ACG TCT TTA GAG GAA CAT AAA GAG Cys Gly Arg Ser Tyr Lys Gln Arg Thr Ser Leu Glu Glu His Lys Glu 225 230 235 240	720
CGC TGC CAC AAC TAC TTG GAA AGC ATG GGC CTT CCG GGC ACA CTG TAC Arg Cys His Asn Tyr Leu Glu Ser Met Gly Leu Pro Gly Thr Leu Tyr 245 250 255	768
CCA GTC ATT AAA GAA GAA ACT AAG CAC AGT GAA ATG GCA GAA GAC CTG Pro Val Ile Lys Glu Glu Thr Lys His Ser Glu Met Ala Glu Asp Leu 260 265 270	816
TGC AAG ATA GGA TCA GAG AGA TCT CTC GTG CTG GAC AGA CTA GCA AGT Cys Lys Ile Gly Ser Glu Arg Ser Leu Val Leu Asp Arg Leu Ala Ser 275 280 285	864
AAT GTC GCC AAA CGT AAG AGC TCT ATG CCT CAG AAA TTT CTT GGG GAC Asn Val Ala Lys Arg Lys Ser Ser Met Pro Gln Lys Phe Leu Gly Asp 290 295 300	912
AAG GGC CTG TCC GAC ACG CCC TAC GAC AGT GCC ACG TAC GAG AAG GAG Lys Gly Leu Ser Asp Thr Pro Tyr Asp Ser Ala Thr Tyr Glu Lys Glu 305 310 315 320	960
AAC GAA ATG ATG AAG TCC CAC GTG ATG GAC CAA GCC ATC AAC AAC GCC Asn Glu Met Met Lys Ser His Val Met Asp Gln Ala Ile Asn Asn Ala 325 330 335	1008
ATC AAC TAC CTG GGG GCC GAG TCC CTG CGC CCG CTG GTG CAG ACG CCC Ile Asn Tyr Leu Gly Ala Glu Ser Leu Arg Pro Leu Val Gln Thr Pro 340 345 350	1056
CCG GGC GGT TCC GAG GTG GTC CCG GTC ATC AGC CCG ATG TAC CAG CTG Pro Gly Gly Ser Glu Val Val Pro Val Ile Ser Pro Met Tyr Gln Leu 355 360 365	1104
CAC AGG CGC TCG GAG GGC ACC CCG CGC TCC AAC CAC TCG GCC CAG GAC His Arg Arg Ser Glu Gly Thr Pro Arg Ser Asn His Ser Ala Gln Asp 370 375 380	1152
AGC GCC GTG GAG TAC CTG CTG CTG CTC TCC AAG GCC AAG TTG GTG CCC Ser Ala Val Glu Tyr Leu Leu Leu Leu Ser Lys Ala Lys Leu Val Pro 385 390 395 400	1200
TCG GAG CGC GAG GCG TCC CCG AGC AAC AGC TGC CAA GAC TCC ACG GAC Ser Glu Arg Glu Ala Ser Pro Ser Asn Ser Cys Gln Asp Ser Thr Asp 405 410 415	1248
ACC GAG AGC AAC AAC GAG GAG CAG CGC AGC GGT CTT ATC TAC CTG ACC Thr Glu Ser Asn Asn Glu Glu Gln Arg Ser Gly Leu Ile Tyr Leu Thr 420 425 430	1296
AAC CAC ATC GCC CGA CGC GCG CAA CGC GTG TCG CTC AAG GAG GAG CAC Asn His Ile Ala Arg Arg Ala Gln Arg Val Ser Leu Lys Glu Glu His 435 440 445	1344

CGC GCC TAC GAC CTC GTG CGC GCC GCC TCC GAG AAC TCG CAG GAC GCG	1392
Arg Ala Tyr Asp Leu Val Arg Ala Ala Ser Glu Asn Ser Gln Asp Ala	
450 455 460	
TTC CGC GTG GTC AGC ACC AGC GGG GAG CAG ATG AAG GTG TAC AAG TGC	1440
Phe Arg Val Val Ser Thr Ser Gly Glu Gln Met Lys Val Tyr Lys Cys	
465 470 475 480	
GAA CAC TGC CGG GTG CTC TTC CTG GAT CAC GTC ATG TAC ACC ATC CAC	1488
Glu His Cys Arg Val Leu Phe Leu Asp His Val Met Tyr Thr Ile His	
485 490 495	
ATG GGC TGC CAC GGC TTC CGT GAT CCT TTT GAG TGC ACC ATG TGC GGC	1536
Met Gly Cys His Gly Phe Arg Asp Pro Phe Glu Cys Thr Met Cys Gly	
500 505 510	
TAC CAC AGC CAG GAC CGG TAC GAG TTC TCG TCG CAC ATA ACG CGA GGG	1584
Tyr His Ser Gln Asp Arg Tyr Glu Phe Ser Ser His Ile Thr Arg Gly	
515 520 525	
GAG CAC CGC TTC CAC ATG ACG TAA GCC	1611
Glu His Arg Phe His Met Thr * Ala	
530 535	

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 38 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AGAAGTTTCC ATAAGATGAT GAATGGGGGT GGCAGAGA

38

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 568 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Asp Val Asp Glu Gly Gln Asp Met Ser Gln Val Ser Gly Lys Glu
 1 5 10 15
 Ser Pro Pro Val Ser Asp Thr Pro Asp Glu Gly Asp Glu Pro Met Pro
 20 25 30
 Val Pro Glu Asp Leu Ser Thr Thr Ser Gly Ala Gln Gln Asn Ser Lys
 35 40 45
 Ser Asp Arg Gly Met Ala Ser Asn Val Lys Val Glu Thr Gln Ser Asp
 50 55 60
 Glu Glu Asn Gly Arg Ala Cys Glu Met Asn Gly Glu Glu Cys Ala Glu
 65 70 75 80
 Asp Leu Arg Met Leu Asp Ala Ser Gly Glu Lys Met Asn Gly Ser His
 85 90 95
 Arg Asp Gln Gly Ser Ser Ala Leu Ser Gly Val Gly Gly Ile Arg Leu
 100 105 110
 Pro Asn Gly Lys Leu Lys Cys Asp Ile Cys Gly Ile Val Cys Ile Gly
 115 120 125
 Pro Asn Val Leu Met Val His Lys Arg Ser His Thr Gly Glu Arg Pro
 130 135 140
 Phe Gln Cys Asn Gln Cys Ser Ser Ala Leu Ser Gly Val Gly Gly Ile
 145 150 155 160
 Arg Leu Pro Asn Gly Lys Leu Lys Cys Asp Ile Cys Gly Ile Val Cys
 165 170 175
 Ile Gly Pro Asn Val Leu Met Val His Lys Arg Ser His Thr Gly Glu
 180 185 190
 Arg Pro Phe Gln Cys Asn Gln Cys Gly Ala Ser Phe Thr Gln Lys Gly
 195 200 205
 Asn Leu Leu Arg His Ile Lys Leu His Ser Gly Glu Lys Pro Phe Lys
 210 215 220
 Cys His Leu Cys Asn Tyr Ala Cys Arg Arg Arg Asp Ala Leu Thr Gly
 225 230 235 240
 His Leu Arg Thr His Ser Val Gly Lys Pro His Lys Cys Gly Tyr Cys
 245 250 255
 Gly Arg Ser Tyr Lys Gln Arg Ser Ser Leu Glu Glu His Lys Glu Arg
 260 265 270
 Cys His Asn Tyr Leu Glu Ser Met Gly Leu Pro Gly Met Tyr Pro Val
 275 280 285
 Ile Lys Glu Glu Thr Asn His Asn Glu Met Ala Glu Asp Leu Cys Lys
 290 295 300

Ile Gly Ala Glu Arg Ser Leu Val Leu Asp Arg Leu Ala Ser Asn Val
 305 310 315 320
 Ala Lys Arg Lys Ser Ser Met Pro Gln Lys Phe Leu Gly Asp Lys Cys
 325 330 335
 Leu Ser Asp Met Pro Tyr Asp Ser Ala Asn Tyr Glu Lys Glu Asp Met
 340 345 350
 Met Thr Ser His Val Met Asp Gln Ala Ile Asn Asn Ala Ile Asn Tyr
 355 360 365
 Leu Gly Ala Glu Ser Leu Arg Pro Leu Val Gln Thr Pro Pro Gly Ser
 370 375 380
 Ser Glu Val Val Pro Val Ile Ser Ser Met Tyr Gln Leu His Lys Pro
 385 390 395 400
 Pro Ser Asp Gly Pro Pro Arg Ser Asn His Ser Ala Gln Asp Ala Val
 405 410 415
 Asp Asn Leu Leu Leu Leu Ser Lys Ala Lys Ser Val Ser Ser Glu Arg
 420 425 430
 Glu Ala Ser Pro Ser Asn Ser Cys Gln Asp Ser Thr Asp Thr Glu Ser
 435 440 445
 Asn Ala Glu Glu Gln Arg Ser Gly Leu Ile Tyr Leu Thr Asn His Ile
 450 455 460
 Asn Pro His Ala Arg Asn Gly Leu Ala Leu Lys Glu Glu Gln Arg Ala
 465 470 475 480
 Tyr Glu Val Leu Arg Ala Ala Ser Glu Asn Ser Gln Asp Ala Phe Arg
 485 490 495
 Val Val Ser Thr Ser Gly Glu Gln Leu Lys Val Tyr Lys Cys Glu His
 500 505 510
 Cys Arg Val Leu Phe Leu Asp His Val Met Tyr Thr Ile His Met Gly
 515 520 525
 Cys His Gly Cys His Gly Phe Arg Asp Pro Phe Glu Cys Asn Met Cys
 530 535 540
 Gly Tyr His Ser Gln Asp Arg Tyr Glu Phe Ser Ser His Ile Thr Arg
 545 550 555 560
 Gly Glu His Arg Tyr His Leu Ser
 565

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 13 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

HNHTGGGAAT DYY

13

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

NNYGGGAAT HNC

13

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TMYGGGAATD YY

12

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGGCGATTTT GGAATTCA CACC

24

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGGCCATGGG AATGAAGGAA CACC

24

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGTGTAATTT GGAATGCTG TGCCT

25

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGGCATGGGA ATGTCTGGAA CACC

24

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGGCATTAAA ATGGGAATAA CACC

24

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGTGTAGGAA TGCGGTAATT GCCT

24

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGTGTGGGAA TAACTGGGAT GCCT

24

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGTGTGGGAA TGTCACTTCA GCCT

24

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 28 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGTGTGGGAA TACTGAGTAT GCCTGCCT

28

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AGGCAAATTT GGGAATACTA CACC

24

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGTGTGTGGG AACATGGGAT GCCT

24

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AGGCCTATTT CCCTTGGGAA CACC

24

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGTGTGGAAC ATCGTGGGAA GCCGCCT

27

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AGGCGCTTGG GAAATTCCAA CACC

24

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AGGCATTCCT AAACCGGGAA CACC

24

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

AGGCACAATT CCTTCGGGAA CACC

24

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGTGTCGGGC TTCGGGAATA GCCT

24

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGTGTTCCAA ACTCGGGAAT GCCT

24

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GGTGTGGAAT CGGGAATTGA GCCT

24

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AGGCTTATCG GGAAACTTA CACC

24

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GGTGTTCCTAA ACGGGGGAAT GCCT

24

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GGTGTGCAAT TCCAAGGAAT GCCT

24

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AGGCGCCATT CCAAGGATAA CACC

24

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AGGCTAATCT TGGAATTCCA CACC

24

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTGTGGACCA TTGGGATGCG CCT

23

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGTGTTCCAA GAATCAGGAT GCCT

24

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ANTTGGGAAT RYY

13

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GGTGACGGT TGGGAATGCG GCCT

24

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GGGTAGGAA TGGGAATACA GCCT

24

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GGTGTGGGA TTGGGAATGT GCCT

24

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GGTGTCGGGA ATTATTTTAG GCCT

24

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GGTGTAACAAA TGGAACAAA GCCT

24

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GGTGTGGGAA AGATATAGCC GCCT

24

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GGTGTTTAAC CAATTGGGAA GCCT

24

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GGTGTTCGG TATTTGGGAA GCCT

24

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GGTGTGGGAT AACTTGGGAA GCCT

24

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

AGGCGGGAAA ACCCATAGGA CACC

24

(2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GGTGTAATCC GTCGGGAACA GCCTA

25

(2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GGCTTTAGAT CAGGGAACAC ACC

23

(2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GGTGTATCCT GGTAGGAATC GCCT

24

(2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

AGGCTATCCC AGGAATTGA CACC

24

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

AGGCAAATTG TTCAGGAACA CACACC

26

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GGTGTCATA AGGAACAATA GCCT

24

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

AGGCAGACCC AAGGAAGCCA CACC

24

(2) INFORMATION FOR SEQ ID NO:53:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

AGGCTATCCC AGGAATTTGA CACC

24

(2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

AGGAGAATCC TATGGGATAC ACC

23

(2) INFORMATION FOR SEQ ID NO:55:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GGTGTTTCATT GGGATAGCAT GCCT

24

(2) INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GGTGTGGGA TTTCTGGATA GCCT

24

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

AGGCGTTTGG GATGTATTTA CACC

24

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GGTGTGGGAT CGCCATATTC

20

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GGTGTGGGAT TGCTTTATTT

20

(2) INFORMATION FOR SEQ ID NO:60:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GGTGTGGGAT TGGGACTAAA GCCTA

25

(2) INFORMATION FOR SEQ ID NO:61:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GGTGTGGGAT TGGGACTAAA GCCT

24

(2) INFORMATION FOR SEQ ID NO:62:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GGTGTAAGGA CAATGGGATA GCCT

24

(2) INFORMATION FOR SEQ ID NO:63:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GGTGTCTAGGA TTTGGGACAC GCCT

24

(2) INFORMATION FOR SEQ ID NO:64:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GGTGTGGGAC TCAAAGAGGC

20

(2) INFORMATION FOR SEQ ID NO:65:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GGTGTCTCTCC AGCGGGATAA GCCT

24

(2) INFORMATION FOR SEQ ID NO:66:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

AGGCATCCGG GATAATAAAA CACC

24

(2) INFORMATION FOR SEQ ID NO:67:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

GGTGTTCTTC GGGATGGCTT GCCT

24

(2) INFORMATION FOR SEQ ID NO:68:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

AGGCTTCACC GGGAGCACGA CACC

24

(2) INFORMATION FOR SEQ ID NO:69:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

GGTGTAGATC CCAGGGATTT GCCT

24

(2) INFORMATION FOR SEQ ID NO:70:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

GGTGTAGGTA GGGACATCCC GCCT

24

(2) INFORMATION FOR SEQ ID NO:71:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GGTGTGAGAA ATAAGGGATA GCCT

24

(2) INFORMATION FOR SEQ ID NO:72:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

AGGCAAAAAA AAGGGGATAA CACC

24

(2) INFORMATION FOR SEQ ID NO:73:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GGTGTGAAAT CTGAGGATCT GAAT

24

(2) INFORMATION FOR SEQ ID NO:74:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

NNTTGGGAWN NC

12

(2) INFORMATION FOR SEQ ID NO:75:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

AGGCTTTTGG GAATACCAGA CACC

24

(2) INFORMATION FOR SEQ ID NO:76:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

AGGCTTGGGA TTGGGAATAA CACC

24

(2) INFORMATION FOR SEQ ID NO:77:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

GGTGTTCTG GGAATGTTG GCCTA

25

(2) INFORMATION FOR SEQ ID NO:78:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

AGGCGTGGGA ATATCAGGAC ACC

23

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

AGGCTGGGAA TGCTGGGAAA CACC

24

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

GGTGTGGGA ATGCTGGAAT GCCTA

25

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

GGTGTAATTG GGAATTTTGA GCCTA

25

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

GGTGTGGGAA AAGTGGGAAT GCCTA

25

(2) INFORMATION FOR SEQ ID NO:83:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

GGTGTTCCTG GGAATGCCAA GCCTA

25

(2) INFORMATION FOR SEQ ID NO:84:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

AGGCTACAGA ATACTGGGAA CACC

24

(2) INFORMATION FOR SEQ ID NO:85:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

AGGCTAAAAA TTCTGGGAA CACC

24

(2) INFORMATION FOR SEQ ID NO:86:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

AGGCATTCCC GTTTTGGGAA CACC

24

(2) INFORMATION FOR SEQ ID NO:87:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

AGGCATTCCC GTTTTGGGAA CACC

24

(2) INFORMATION FOR SEQ ID NO:88:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

GGTGTATCCC GGAATACCG GCCTA

25

(2) INFORMATION FOR SEQ ID NO:89:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

AGGCTAAGGA ATACCGGGAA CACC

24

(2) INFORMATION FOR SEQ ID NO:90:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

AGGCTCTGGA ATATCGGGAA CACC

24

(2) INFORMATION FOR SEQ ID NO:91:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

GGTGTAATC GGGAATTCCG GCCTA

25

(2) INFORMATION FOR SEQ ID NO:92:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

AGGCCGGGAA TACCGGAAAA CACC

24

(2) INFORMATION FOR SEQ ID NO:93:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

AGGCAAAACA TTACAGGGAA CACC

24

(2) INFORMATION FOR SEQ ID NO:94:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

AGGCAGGGAA TATCGGGATA CACC

24

(2) INFORMATION FOR SEQ ID NO:95:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

GGTGTAGGAA TTCTAGGAAT GCCTA

25

(2) INFORMATION FOR SEQ ID NO:96:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

AGGCATTCCA AGGAATTTTA CACC

24

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

GGTGTAAGGA ATACTGGAAT GCCTA

25

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

GGCAGAATTC CAAGGAATAC ACC

23

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

AGGCCAAGGA ATATCAGGAA CACC

24

(2) INFORMATION FOR SEQ ID NO:100:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

TNHTGGGAAT DYY

13

(2) INFORMATION FOR SEQ ID NO:101:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

TTTTGGGAAT ACC

13

(2) INFORMATION FOR SEQ ID NO:102:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

TCAGCTTTTG GGAATCTCCT GTCA

24

(2) INFORMATION FOR SEQ ID NO:103:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

TGGAGGGAAG TGGGAACTT TT

22

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

TGGAAGTGGG AGGC

14

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

GAGGAGAAAG GTCTCCTAC

19

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

AACAGGGAAA CA

12

(2) INFORMATION FOR SEQ ID NO:107:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

GTCAGGGAAC AGG

13

(2) INFORMATION FOR SEQ ID NO:108:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

AAGGTGGGAA GTAA

14

(2) INFORMATION FOR SEQ ID NO:109:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 11 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

GGTAGGAATG G

11

(2) INFORMATION FOR SEQ ID NO:110:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

GGAGGGGGAA GAA

13

(2) INFORMATION FOR SEQ ID NO:111:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

AGTGGGGAAA TCT

13

(2) INFORMATION FOR SEQ ID NO:112:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

GGTCAGGGAA ACAA

14

(2) INFORMATION FOR SEQ ID NO:113:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

TGGGGGAAGG GGTGAAG

18

(2) INFORMATION FOR SEQ ID NO:114:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 11 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

TTTTGGGAAC C

11

(2) INFORMATION FOR SEQ ID NO:115:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

AAAGGGGAAC CC

12

(2) INFORMATION FOR SEQ ID NO:116:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

TGGAGGGGAG

9

(2) INFORMATION FOR SEQ ID NO:117:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

AGGGGAAA

8

(2) INFORMATION FOR SEQ ID NO:118:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

TTTGGGAATT

10

(2) INFORMATION FOR SEQ ID NO:119:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 16 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

TGAGAGGAAG AGGAGA

16

(2) INFORMATION FOR SEQ ID NO:120:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

CAGGAATT

8

(2) INFORMATION FOR SEQ ID NO:121:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

AAGGAAACCA AACAGGGGA AG

22

(2) INFORMATION FOR SEQ ID NO:122:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

TTGGAAACCT

10

(2) INFORMATION FOR SEQ ID NO:123:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

GTTTCCATGA CATCATGAAT GGGAGT

26

(2) INFORMATION FOR SEQ ID NO:124:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

GTTTCCATGA TGTATGAAT GGGGGT

26

(2) INFORMATION FOR SEQ ID NO:125:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

TTCTTGGGGA TTG

13

(2) INFORMATION FOR SEQ ID NO:126:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

GGAGGAACT

9

(2) INFORMATION FOR SEQ ID NO:127:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

TTTGGGATG

9

(2) INFORMATION FOR SEQ ID NO:128:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

TTCTAGGAAG TAAGGAATT T

21

(2) INFORMATION FOR SEQ ID NO:129:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

GTGGGAAGA

9

(2) INFORMATION FOR SEQ ID NO:130:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

TAGGAATTCT

10

(2) INFORMATION FOR SEQ ID NO:131:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

TAAGGAAAGG

10

(2) INFORMATION FOR SEQ ID NO:132:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

TTTCCAAGTG GGAATC

16

(2) INFORMATION FOR SEQ ID NO:133:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

TGGGGAGTT

9

(2) INFORMATION FOR SEQ ID NO:134:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 11 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

TTGGGAAGGA T

11

(2) INFORMATION FOR SEQ ID NO:135:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

AAGGAACA

8

(2) INFORMATION FOR SEQ ID NO:136:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

CAGGGGAATC TCCCTCTCCA T

21

(2) INFORMATION FOR SEQ ID NO:137:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

AAGAGGAAAA

10

(2) INFORMATION FOR SEQ ID NO:138:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

GGGAAATTCC

10

(2) INFORMATION FOR SEQ ID NO:139:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

GGGGAATCCC

10

(2) INFORMATION FOR SEQ ID NO:140:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

TGGGAG

6

(2) INFORMATION FOR SEQ ID NO:141:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

CAGGGAAGTA

10

(2) INFORMATION FOR SEQ ID NO:142:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 36 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

CAAGGGACTT TCCGCTGGGG ACTTTCAGG GAGGCG

36

(2) INFORMATION FOR SEQ ID NO:143:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 103 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

TTTGGTTATA AATGTATTGA TTGCATCCCC ATTACCCAGA AGGCCAATAT TTAATTGGAG

60

TCTTAACTCA ATTGTGTTTT CGTCAGTTGG TAAGCCTCAC AAA

103

(2) INFORMATION FOR SEQ ID NO:144:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 116 base pairs
 - (B) TYPE: nucleic acid

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

```
ATGGGCCTTC CGGGCATGTA CCCAGGTAAG CACTGAGGCC CTGCTGAGCT GCACCCCTCC      60
CCCTCCCAGC GCCTGGGCCA GGATGGGGCT CTGTGGCCTG TTTCAGCCAC AGGAGG      116
```

(2) INFORMATION FOR SEQ ID NO:145:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 94 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:

```
CCTTGTTGCT GCTGTGTTGC TATCTTGTA CTTATTTTTC CAGTGACACT GAGTGGCCTC      60
CTGTGTTGTC TCTTTCAGCC AGTAATGTTA AAGT      94
```

(2) INFORMATION FOR SEQ ID NO:146:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 120 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:

GAGCCCTGGC AGATGTGTCC TGTCTGCTGT GACACTAGAA CACCATTCAA CCCCTGGGTG 60
TAGATTTTAC TTATGACCAT CTACTTCCCG CAGGAGACAA GTGCCTGTCA GACATGCCCT 120

(2) INFORMATION FOR SEQ ID NO:147:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 120 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:

ACATGTGTGG TTATCACAGC CAGGACAGGT ACGAGTTCTC ATCCCATATC ACGCGGGGGG 60
AGCATCGTTA CCACCTGAGC TAAACCCAGC CAGGCCCCAC TGAAGCACAA AGATAGCTGG 120

(2) INFORMATION FOR SEQ ID NO:148:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

AGGAGGAAAA 10

(2) INFORMATION FOR SEQ ID NO:149:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:149

TGGGAAT 7

(2) INFORMATION FOR SEQ ID NO:150:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

TCAGCTTTTG GGAATCTCCT GTCA

24

(2) INFORMATION FOR SEQ ID NO:151:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

TCAGCTTTTG GGATTCCTCT CA

22

(2) INFORMATION FOR SEQ ID NO:152:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

TCAGCGGGGG GGAATACCCT GTCA

24

CLAIMS:

1. A purified DNA comprising a sequence which encodes a peptide comprising an Ikaros exon.
2. The purified DNA of claim 1, wherein said peptide further encodes a second Ikaros exon.
3. The purified DNA of claim 2, wherein said peptide further encodes a third Ikaros exon.
4. The purified DNA of claim 3, wherein said peptide further encodes a fourth Ikaros exon.
5. The purified DNA of claim 4, wherein said peptide further encodes a fifth Ikaros exon.
6. The purified DNA of claim 5, wherein said peptide further encodes a sixth Ikaros exon.
7. A purified peptide comprising an Ikaros exon.
8. The purified peptide of claim 7, further comprising a second Ikaros exon.
9. The purified peptide of claim 8, further comprising a third Ikaros exon.
10. The purified peptide of claim 9 further comprising a fourth Ikaros exon.
11. The purified peptide of claim 10, further comprising a sequence which encodes a fifth Ikaros exon.
12. The purified peptide of claim 11, further comprising a sequence which encodes a sixth Ikaros exon.
13. Purified DNA comprising a sequence at least 85% homologous with DNA from SEQ ID NO:2 or SEQ ID NO:3 and encoding a peptide having Ikaros activity.
14. Purified DNA comprising a sequence encoding a peptide of 40 or more amino acids in length and having at least 90 % homology with an amino acid sequence

which is the same, or essentially the same, as the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:3.

15. A purified peptide having Ikaros activity and 85% homologous with a naturally occurring Ikaros isoform.
 16. A vector comprising a DNA sequence encoding an Ikaros peptide.
 17. A cell containing the purified DNA of claim 16.
 18. A method for manufacture of an Ikaros peptide comprising culturing the cell of claim 17 in a medium to express said Ikaros peptide.
 19. A substantially pure preparation of an antibody directed against an Ikaros peptide.
 20. A therapeutic composition comprising an Ikaros peptide and a pharmaceutically acceptable carrier.
 21. A method for treating an animal having an immune system disorder, comprising administering a therapeutically-effective amount of an Ikaros peptide to said animal.
 22. A method of treating an animal having an immune system disorder, comprising administering to said animal a cell selected for the expression of a product of the Ikaros gene.
 23. A method for treating an animal having an immune system disorder, comprising administering to said animal a nucleic acid encoding an Ikaros peptide and expressing said nucleic acid.
 24. A method of evaluating the effect of a treatment comprising carrying out said treatment and evaluating the effect of said treatment on the expression of the Ikaros gene.
 25. A method for determining if a subject is at risk for an immune disorder comprising examining said subject for the expression of the Ikaros gene, mis-expression being indicative of risk.
-

26. A method for determining if a subject is at risk for an immune disorder comprising providing a nucleic acid sample from said subject and determining if the structure of the Ikaros gene differs from wild type.

27. A method of evaluating an animal or cell model for an immune disorder comprising determining if the Ikaros gene in said animal or cell model is expressed at a predetermined level.

28. A transgenic rodent having a transgene which comprises an Ikaros gene.

29. A method of expressing a heterologous gene comprising placing said gene under the control of an Ikaros-responsive control element, and contacting said Ikaros-responsive control element with an Ikaros peptide.

30. A method of expressing a gene under the control of an Ikaros-responsive control element in a cell comprising administering an Ikaros peptide to said cell.

31. A method for treating an animal having a disorder of the corpus striatum, comprising administering a therapeutically-effective amount of an Ikaros protein to said animal.

32. A method of treating an animal having a disorder of the corpus striatum, comprising administering to said animal a cell selected for the expression of a product of the Ikaros gene.

33. A method for treating an animal having a disorder of the corpus striatum, comprising administering to said animal a nucleic acid encoding Ikaros and expressing said nucleic acid.

34. A method for determining if a subject is at risk for a disorder of the corpus striatum, comprising examining said subject for the expression of the Ikaros gene, mis-expression being indicative of risk.

35. A method for determining if a subject is at risk for a disorder of the corpus striatum, comprising providing a nucleic acid sample from said subject and determining if the structure of the Ikaros gene differs from wild type.

36. A method of evaluating an animal or cell model for a disorder of the corpus striatum, comprising determining if the Ikaros gene in said animal or cell model is misexpressed.

37. A method of inhibiting the binding of a first Ikaros isoform, and a DNA sequence, comprising contacting said DNA sequence with an effective amount of a second Ikaros isoform, or with a DNA binding fragment of an Ikaros isoform.

38. A method of inhibiting binding between a first Ikaros isoform, and a DNA sequence, comprising contacting said peptide with an effective amount of an Ikaros binding oligonucleotide.

39. An Ikaros binding oligonucleotide.

40. A method of attenuating the binding of a first Ikaros isoform to target DNA comprising contacting said target DNA with an effective amount of a second Ikaros isoform, or with a DNA binding fragment of said second isoform.

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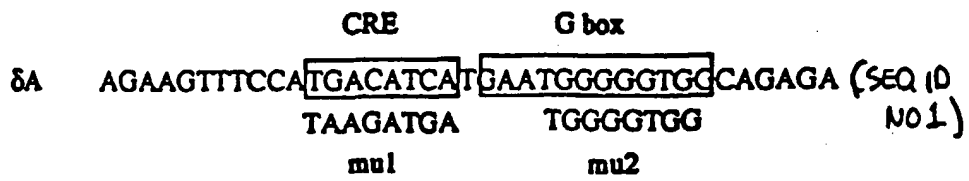


FIG. 1A

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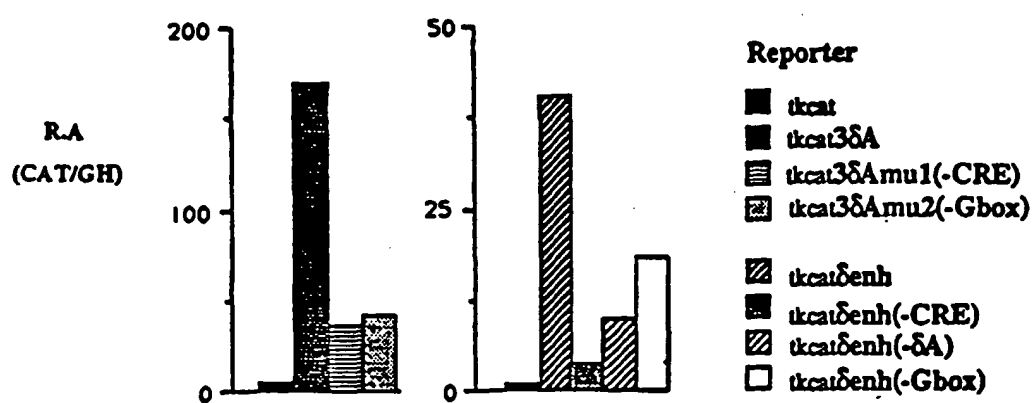


FIG. 1B

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FIG. 1C

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1
AAT TCC TTC TAC CTT CTC TGA ACC CCA CTG 31
61 /
GAA CAG CAA CAG CAA CAG CAA TCT CCG OCT 91
121
CTA CTC AAG CCA CAC TCA CAG CAA CTC TAG ATT TGT GTG OCA CAG AGA CAC AOC TCT COT
181
TTC GCC TTG CCG ACG CAC AAG TCT GTT GAT AAC CTG AAG ACA ATG CAT CTC GAT CAG CGT
241
CAA CAC ATG TCC CAA GTT TCA GCA AAG GAG AOC CCG CCA CTC AGT CAC ACT CCA CAT CAA
301 / 101
GCC CAT gag ccc atg cct gtc cct CAC CAC CTG TCC act acc tct gga gca cag cag aac
361 / 121
tcc aag aat GAT CGA CCG ATG GGT CAA CCG CCT TTC CAG TOC AAC CAG TOT CCG OCC TCC
421 / 141
TTT ACC CAG AAA CCG AAC CTC CTG CCG CAC ATC AAG CTG CAC TCC GGT CAG AAG CCC TTC
481 / 161
AAA TOC CAT CTT TCC AAC TAT CCG TCC CCG CCG AGG CAC CCG CTC ACC CCG CAC CTG ACG
541 / 181
ACG CAC TCC GTT CGT AAG CCT CAC AAA TGT GCA TAT TGT CCG CCG AOC TAT AAA CAG CCA
601 / 201
ACG TCT TTA GAG CAG CAT AAA CAG CGA TOC CAC AAC TAC TTA GAA AOC ATG CCG CTT CCG
661 / 221
CGC CTG TCC CCA CTC ATT AAG CAA GAA ACT AAC CAC AAG CAG ATG CCA CAA CAC CTG TCC
721 / 241
AAG ATA CCA CCA CAG ACG TCC CTT CTC CTG CAC ACG CTG CCA AOC AAT CTC CCG AAA COT
781 / 261
AAG AOC TCT ATG CCG CAG AAA TTT CTT GCA CAC AAG TOC CTG TCA CAC ATG CCG TAT CAC
841 / 281
AGT CCG AAC TAT CAG AAG CAG CAT ATG ATG ACA TCC CAC CTG ATG CAC CAG CCG ATC AAC
901 / 301
AAT CCG ATC AAC TAC CTG CCG OCT CAG TCC CTG CCG CCA TTA TTA CAG ACA CCG CCG COT
961 / 321
AOC TCC CAG CTG GTG CCA CTC ATC AOC TCC ATG TAC CAG CTG CAC AAG CCG CCG TCA CAT
1021 / 341
CGC CCG CCA CCG TCC AAC CAT TCA CCA CAG CAC CCG CTG GAT AAC TTA CTG CTG CCG TCC
1081 / 361
AAG CCG AAG TCT CTG TCA TCG CAG CCA CAG CCG TCC CCG AOC AAC AOC TCC CAA CAC TCC
1141 / 381
ACA GAT ACA CAG AOC AAC CCG CAG GAA CAG CCG AOC CCG CTT ATC TAC CTA ACC AAC CAC
1201 / 401
ATC AAC CCG CAT CCA CCG AAT CCG CTG OCT CTC AAG CAG CAG CCG CCG TAC CAG CTG
1261 / 421
CTC ACG CCG CCG TCA CAG AAC TCG CAG CAT CCG TTC CCG CTC CTC AOC AOC ACT CCG CAG

FIG 2

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```

leu arg ala ala ser glu asn ser gln asp ala phe arg val val ser thr ser gly glu
1 / 441 1351 / 451
CAG CTO AAG GTO TAC AAG TOC GAA CAC TOC COC GTO CTC TTC CTO GAT CAC GTC ATG TAT
jln leu lys val tyr lys cys glu his cys arg val leu phe leu asp his val met tyr
1381 / 461 1411 / 471
ACC ATT CAC ATG GOC TOC CAT GOC TOC CAT COC TTT COG GAT CCC TTT GAG TOT AAC ATG
thr ile his met gly cys his gly cys his gly phe arg asp pro phe glu cys asn met
1441 / 481 1471 / 491
TOT GGT TAT CAC AOC CAG GAC AOC TAC GAG TTC TCA TOC CAT ATC ACG GCG GCG GAG CAT
cys gly tyr his ser gln asp arg tyr glu phe ser ser his ile thr arg gly glu his
1501 / 501 1531 / 511
CGT TAC CAC CTO AOC TAA ACC CAG CCA COC COC ACT GAA GCA CAA AGA TAG CTC GTT ATG
arg tyr his leu ser
1561 / 521 1591 / 531
CCT CCT TCC COG CAG CTG GAC CCA CAG COG ACA ATC TOG GAG TCG ATT TOC ACG CAG CAT
531 / 541 1651 / 551
TTG TTC TTT TAT GTT GGT TGT TTG GCG TTT GAT TTG CCT TGG AAG ATA AGT TTT TAA TGT
1681 / 561 1711 / 571
TAG TCA CAG GAT TOC ATT GCA TCA GCA ACA TTC ACA ACA TOC ATC CTT CTA GCG AGT TTT
1741 / 581 1771 / 591
GTT CAC TOG TAG CTG ACG TTT CCC GCA TAT GTG OCT TCC TAA CAC TCT

```

(SEQ ID No2)

Fig 2 cont

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1 / 1
 GAA TTC CCG CTT TGG GCG CTG acc 999 ACC CCG CCG c9A TTG Caa AGT TTT CGT GCG CGC
 glu phe arg leu trp gly leu thr gly ser pro ala arg leu gln ser phe arg ala arg
 61 / 21
 CCC TCT GGC CCG GAG TTG CCG CTG AGA c9c 9cc 9cc 999 99A CTC GGC GAC GGG
 pro ser gly pro glu leu arg leu arg arg ala ala arg ala gly gly leu gly asp gly
 121 / 41
 CCG GCG ACG GGA CGA CCG ACC CTC TCC GTG TCC GCT CTC GCC CTT CTG CCG GCC CCG CTC
 ala gly thr gly arg arg thr leu ser val ser ala leu ala leu leu arg ala pro leu
 181 / 61
 CCT GTA CCG GAG CAG CGA, TCC GGG ACG CCG CCG AGA Ccc agt aat gtt AAA GTA GAG ACT
 pro val pro glu gln arg ser gly arg arg pro arg ala ser asn val lys val glu thr
 241 / 81
 CAG AGT GAT GAA GAG AAT GGG CGT GcC TGT GAA ATG AAT GGG GAA GAA TGT GCG GAG GAT
 gln ser asp glu glu asn gly arg ala cys glu met^{met} asn gly glu glu cys ala glu asp
 301 / 101
 TTA cga atg ctt gat gcc tcg gga gag aaa atg aat ggc tcc cac agG gac caa ggc agc
 leu arg met leu asp ala ser gly glu lys met asn gly ser his arg asp gln gly ser
 361 / 121
 tcg gct ttg tcg gga gtt q9a ggc att cga ctt cct aac gga aaa cta aag tgt gat atc
 ser ala leu ser gly val gly gly ile arg leu pro asn gly lys leu lys cys asp ile
 421 / 141
 tgt g9g atc att tgc atc g9g ccc aat gtg ctc atg gtt cac aaa aga agc cac act gga
 cys gly ile ile cys ile gly pro asn val leu met val his lys arg ser his thr gly
 481 / 161
 gaa c9g ccc ttc cag tgc aat cag tgc g9g gcc tca ttc acc cag aag Ggc aac Ctg ctc
 glu arg pro phe gln cys asn gln cys gly ala ser phe thr gln lys gly asn leu leu

FIG. 3

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541 / 181 571 / 191
 cgg cac atc aag ctg cat tcc ggg gag aag ccc ttc aaa tgc cac ctg tgc aac tac gcc
 arg his ile lys leu his ser gly glu lys pro phe lys cys his leu cys asn tyr ala
 601 / 201 631 / 211
 tgc cgc cgg aqg gac gcc ctg act ggc cac ctg agg acg cac tcc gtt ggt aaa cct cac
 cys arg arg arg asp ala leu thr gly his leu arg thr his ser val gly lys pro his
 661 / 221 691 / 231
 aaa tgt gga tat tgt ggc cga agc tat aAa cag cga acg tct tta gag gaa cat aaa gag
 lys cys gly tyr cys gly arg ser tyr lys gln arg thr ser leu glu glu his lys glu
 721 / 241 751 / 251
 cgc tgc cac aac tac ttg gaa agc atg ggc ctt ccg ggc aca ctg tac cca gtc att aaa
 arg cys his asn tyr leu glu ser met gly leu pro gly thr leu tyr pro val ile lys
 781 / 261 811 / 271
 gaa gaa act aag cac agt gaa atg gca gaa gac ctg tgc aag ata gga tca gag aga tct
 glu glu thr lys his ser glu met ala glu asp leu cys lys ile gly ser glu arg ser
 841 / 281 871 / 291
 ctg ctg ctg gac aga cta gca agt aaf gtc gcc aaa cgt aag agc tct atg cct cag aaa
 leu val leu asp arg leu ala ser asn val ala lys arg lys ser ser met pro gin lys
 901 / 301 931 / 311
 ttt ctt gng gac aag ggc ctg tcc gac acg ccc tac gac agt gcc acg tac gag aag gag
 phe leu gly asp lys gly leu ser asp thr pro tyr asp ser ala thr tyr glu lys glu
 961 / 321 991 / 331
 aac gaa atg atg aag tcc cac ctg atg gac caa gcc atc aac aac gcc atc aac TAC CTg
 asn glu met met lys ser his val met asp gln ala ile asn asn ala ile asn tyr leu
 1021 / 341 1051 / 351
 qgc gcc gag tcc ctg cgc ccg ctg ctg gag acg ccc ccg ggc ggt tcc gag gtc ccg
 gly ala glu ser leu arg pro leu val gln thr pro pro gly ser glu val val pro

F16.3 cont

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1081 / 361      1111 / 371
gtc atc agc ccg atg tac cag ctg cac agc cgc tcg gag ggc acc ccg cgc tcc aac cac
val ile ser pro met tyr gln leu his arg arg ser glu gly thr pro arg ser asn his
1141 / 381      1171 / 391
tcg gcc cag gac agc gcc gtg gag tac ctg ctg ctg tcc aag gcc aag ttg ttg ccc
ser ala gln asp ser ala val glu tyr leu leu leu leu ser lys ala lys leu val pro
1201 / 401      1231 / 411
tcg gag cgc gag cgc gcc cgc agc aac agc tgc caa gac tcc acg gac acc gag agc aac
ser glu arg glu ala ser pro ser asn ser cys gln asp ser thr asp thr glu ser asn
1261 / 421      1291 / 431
aac gag gag cag cgc agc ggt ctt atc tac ctg acc aac cac atc gcc cga cgc gcg caa
asn glu glu gln arg ser gly leu ile tyr leu thr asn his ile ala arg arg ala gln
1321 / 441      1351 / 451
cgc gtg tcg ctc aag gag gag cac cgc gcc tac gac ctc gtg cgc gcc gcc tcc gag aac
arg val ser leu lys glu glu his arg ala tyr asp leu val arg ala ala ser glu asn
1381 / 461      1411 / 471
tcg cag gac cgc ttc cgc gtg gtc agc acc agc ggg gag cag atg aag gtg tac aag tgc
ser gln asp ala phe arg val val ser thr ser gly glu gln met lys val tyr lys cys
1441 / 481      1471 / 491
gaa cac tgc cgg gtc ctc ttc ctg gat cac gtc atg tac acc atc cac atg gcc tgc cac
glu his cys arg val leu phe leu asp his val met tyr thr ile his met gly cys his
1501 / 501      1531 / 511
ggc ttc cgt gat cct ttt gag tgc aac atg tgc gcc tac cac agc cag gac cgg tac gag
gly phe arg asp pro phe glu cys asn met cys gly tyr his ser gln asp arg tyr glu
1561 / 521      1591 / 531
ttc tcg tcg cac ata acg cga ggg gag cac cgc ttc cac atg acg taa gcc
phe ser ser his ile thr arg gly glu his arg phe his met thr och ala

```

FLU: 3 CONT

(SEQ 10 NO: 3)

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▼ Ex1/2 MDVDEGQDMS QVSGKESPPV SDTPDEGDEP MPVPEDLSTT SGAQQNSKSD RGMASNVKVE ▼ Ex3 TQSDENGRA CEMNGEECAE DLRLDASGE
 KMNGSHRDQ SSALSGVGGI RLPNGKLKCD ICGIVCIGPN VLMVHKRSHT GERPFQCNQC ▼ Ex4 SSALSGVGGI RLPNGKLKCD ICGIVCIGPN
 VLMVHKRSHT GERPFQCNQC GASFTQKGNL LRHIKLHSGE KPFKCHLCNY ACRRRDALTG HLRTHSVGKP ▼ Ex5 HKCGYCGRSY KQSSLEEHHK
 ERCHNYLES GLPGMYPVIK ▼ Ex6 EETNHNEAE DLCKIGAERS LVLDRLASNV AKRKSSMPQK FLGDKCLSDM PYDSANYEKE DMMTSHVMDQ
 AINNAINYLG AESLRPLVQT PPGSSEVPV ISSMYQLHKP PSDGPPRSNH SAQDAVDNLL LLSKAKSVSS EREAAPSNSC QDSTDTESNA
 EEQRSGLIYL TNHINPHARN GLALKEEQRA YEVLRAASEN SQDAFRVVST SGEQLKVYKC EHCRLVFLDH VMYTHMGCH GCHGFRDPFE
 CNMCGYHSQD RYEFSSHITR GEHRYHLS (SEQ ID NO 5)

FIG. 4

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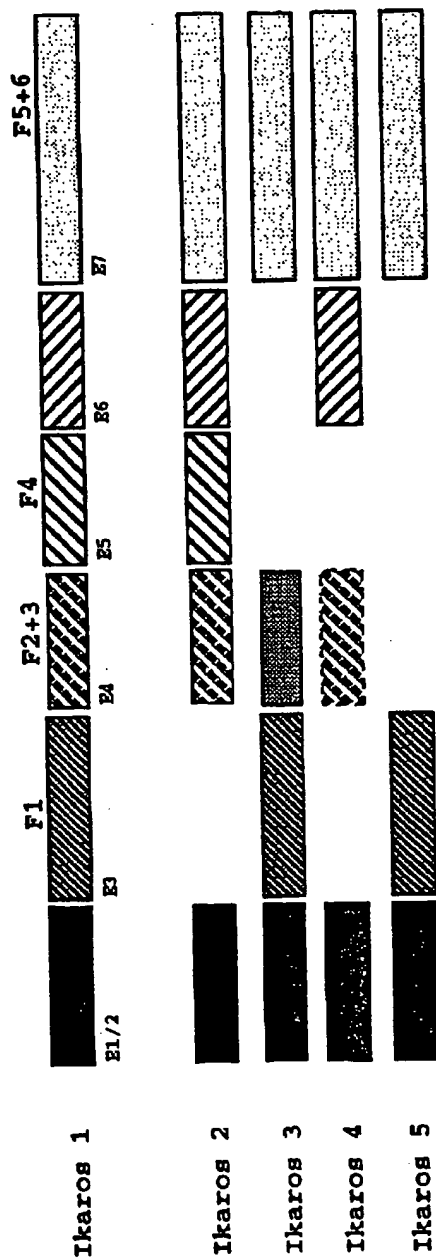


FIG 5

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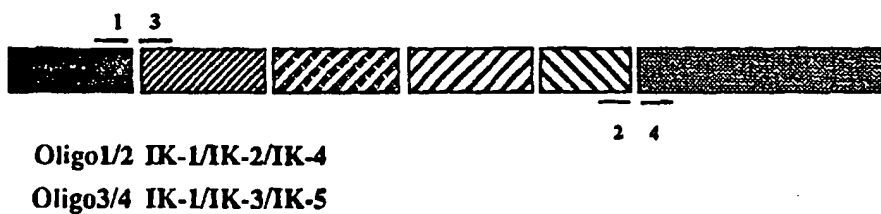


FIG 6



FIG. 7

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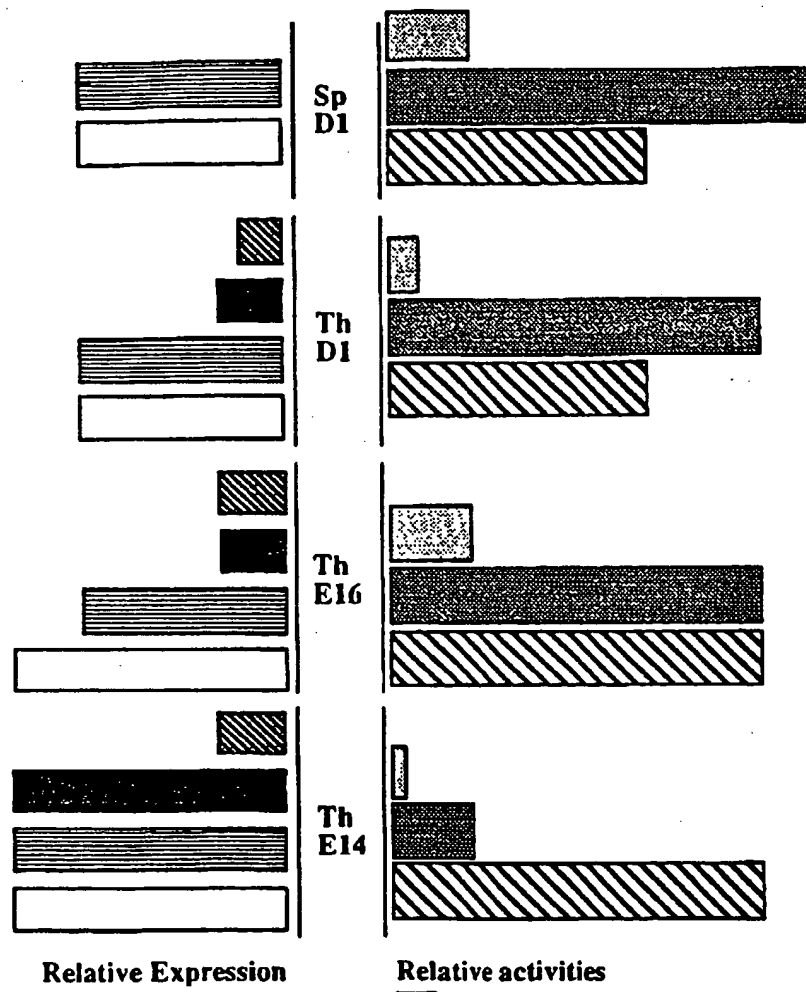


FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08743

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C07H 21/00

US CL : 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, "Ikaros protein"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	No relevant art found.	

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

•	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"Z"	document member of the same patent family

Date of the actual completion of the international search

03 DECEMBER 1993

Date of mailing of the international search report

DEC 28 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

Authorized officer

SUZANNE ZISKA

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

Int. l. application No.
PCT/US93/08743

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
(Form PCT/ISA/206 Previously Mailed.)
Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6, 13, 14, 16-18

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08743

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

See written lack of unity, mailed 11/15/93. Attorney had not responded, but when telephoned, stated that Applicants would not pay for additional groups.

Group I, consisting of a first product, claims 1-6, 12, 14, 16-18, drawn to a DNA and a first method of using the first product, involving manufacture of the Ikaros protein using the transfected cell; classified in Class 435, subclass 172.3, for example;

Group II, consisting of a second product, claims 7-13 and 15, drawn to a peptide, classified in Class 530, subclass 350, for example;

Group III, claim 19, consisting of a third product, drawn to an antibody to the Ikaros peptide, classified in Class 530, subclass 387, for example;

Group IV, consisting of a first method of using the second product, claims 20, 21 and 31, drawn to a protein in a therapeutic composition and a method of therapy, classified in Class 514, subclass 2, for example;

Group V, claims 22 and 32, consisting of a second method, drawn to a method of therapy comprising administering a cell selected for the expression of a product of the Ikaros gene, classified in Class 424, subclass 93+, for example;

Group VI, claims 23 and 33, consisting of a second method of using the first product, drawn to a method or therapy comprising administering nucleic acid encoding an Ikaros peptide, classified in Class 514, subclass 44, for example;

Group VII, consisting of a third method of using the first product, claims 24-27, 34-36, drawn to a method of evaluating the effect of treatment comprising evaluating the effect of said treatment on the expression of the Ikaros gene, classified in Class 435, subclass 7.2+, for example;

Group VIII, consisting of a fifth product, claim 28, drawn to a transgenic rodent, classified in Class 800, subclass 2, for example;

Group IX, consisting of a third method, claims 29 and 30, drawn to a method of expressing a heterologous gene, classified in Class 435, subclass 172.3, for example;

Group X, consisting of a fourth method, claims 37-40, drawn to methods of inhibiting binding, classified in Class 536, subclass 23.1, for example.

In the examination of international applications filed under the Patent Cooperation Treaty, PCT Rule 13.1 states that the international application shall relate to one invention only or to a group of inventions so linked as to form "single general inventive concept".

PCT Rule 13.2 indicates that this shall be construed as permitting, in particular, one of the following three possible combinations of the claimed invention:

- (1) a product, a process specifically adapted for the manufacture of said product and a use of said product, or
- (2) a process, and an apparatus or means specifically designed for carrying out said process, or
- (3) a product, a process specially adapted for the manufacture of said product and an apparatus or means designed for carrying out the process.

Additionally, current United States Patent and Trademark

Office restriction practice permits the

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08743

following
combinations of the claimed invention:

- (4) a product, and a process specifically adapted for the manufacture of said product, and
- (5) a product, and a use of the said product, as where said use as claimed cannot be practiced with another materially different product.

The inventions listed as Groups I-X do not meet the requirements for Unity of Invention for the following reasons:

and it considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below:

Each grouping of claims forms a separate invention not linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept. Note that PCT Rule 13 does not provide for multiple products and methods within a single application.

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